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Canadian Environmental Quality Guidelines for Nonylphenol and its Ethoxylates

Report No. 1-3

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This particular issue provides the scientific supporting information and rationale for the development of Canadian Environmental Quality Guidelines for nonylphenol and its ethoxylates. The information in this document is current as of April 2001. Minor revisions and editorial changes have been made for publication in 2002. For additional information regarding these guidelines, please contact:

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Canadian Water and Sediment Quality Guidelines are approved by the Water Quality Task Group of the Canadian Council of Ministers of the Environment (CCME), and Canadian Soil Quality Guidelines are approved by the CCME Soil Quality Guidelines Task Group. Environment Canada acts as the federal representative and serves as the technical secretariat to both of these Task Groups. These guidelines are included as a 2002 update to the Canadian Environmental Quality Guidelines, which was published by the CCME in October of 1999. For CCME publications, please contact:

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This scientific supporting document is available in English only. Factsheets are also available in English under the title *Canadian Environmental Quality Guidelines* (CCME 1999). Ce document scientifique du soutien n'est disponible qu'en anglais avec un résumé en français. Des feuillets d'information sont aussi disponibles en français sous le titre *Recommandations canadiennes pour la qualité de l'environnement* (CCME 1999).

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EXECUTIVE SUMMARY

This scientific supporting document provides the rationale for the development of Canadian Environmental Quality Guidelines for nonylphenol (NP) and nonylphenol ethoxylates (NPEs). It contains a review of technical background information on the chemical and physical properties of NP and NPEs, a review of sources and emissions in Canada, the distribution and behaviour of NP and NPEs in the environment, and their toxicological effects on a variety of aquatic and terrestrial species. This information is used to derive water quality, sediment quality, and soil quality guidelines for NP and NPEs to protect ecological receptors in each of these environmental compartments. All of the Canadian Environmental Quality Guidelines in this document are based on the best available toxicity data at the time of writing. The most recent literature search was conducted in August 2000, but some information obtained since that date has also been included in response to reviewers' comments.

NPEs are a group of nonionic organic chemicals that are used as surfactants, detergents, emulsifiers, wetting agents, and dispersants in a wide variety of industrial processes and commercial products. They are primarily released to the environment through sewage effluents or industrial wastewaters. In sewage treatment systems, and upon entering the environment, NPEs readily undergo an initial primary degradation which largely involves the loss of ethoxylate groups. This results in the formation of more refractory degradation products such as nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxy acetic acid (NP1EC), and nonylphenoxy ethoxy acetic acid (NP2EC).

The major pathway for nonylphenolic substances to enter the environment is through the discharge of municipal and industrial wastewaters into surface waters. Because they are used as formulants for some pesticides, nonylphenolic substances may also be deposited on surface waters through aerial spraying of pesticides, or as runoff from agricultural land or forests which have been sprayed. Once in the aquatic environment, the more hydrophobic chemicals, such as NP, will tend to adsorb to particulates and accumulate in bottom sediments. In wastewater treatment plants, NP will also partition to the sludge, and may then become a contaminant of terrestrial systems when the sludge is landfilled or applied as a soil amendment to agricultural soils. Leaching of nonylphenolic substances into groundwater has also been documented.

It has been proposed that nonylphenol and its ethoxylates be designated as "CEPA toxic" under the Canadian Environmental Protection Act (CEPA 1999). Large quantities of NPEs are used in Canada each year. Therefore, their degradation products (i.e., NP and short-chain NPEs), which are more persistent, toxic, and estrogenic, may be released at concentrations that could affect aquatic and terrestrial life in the receiving environments. In light of these possible impacts, the development of Canadian Environmental Quality Guidelines (CEQGs) has been deemed necessary. CEQGs are nationally-endorsed, science-based goals that are recommended as levels that should result in negligible risk to biota and ecosystem health. These guidelines are developed according to the procedures described in nationally approved protocols for the derivation of Canadian Environmental Quality Guidelines (CCME 1991; CCME 1995; CCME 1996).

A summary of the Canadian Environmental Quality Guidelines developed in this document is provided in Table 1. Guidelines in this document were developed based on data for nonylphenol, but, as described in Chapter 3, it is recommended that a toxic equivalency (TEQ) approach be used to apply these guidelines to mixtures of nonylphenolic substances. When comparing an environmental sample to the guidelines, concentrations of each nonylphenolic substance in the sample should be multiplied by their respective toxic equivalency factors (TEFs) and then summed to yield a total concentration in NP TEQ units. Based on the toxicity of nonylphenol ethoxylates and nonylphenoxy carboxylates relative to nonylphenol, an initial set of TEFs has been recommended. Future work to develop and validate ethoxamer-specific TEF values is needed, but, in the interim, it is recommended that the TEFs presented here be applied.

Tissue residue guidelines in aquatic biota for the protection of wildlife consumers of aquatic life have not been derived due to the lack of available toxicity data for mammalian and avian species.

Sufficient toxicity data were available for the derivation of a full freshwater water quality guideline, but not for a marine water quality guideline. The freshwater guideline was based on the most sensitive chronic endpoint from a primary study, which was a 91-day lowest observed effect concentration (LOEC) for growth reduction in rainbow trout at a nonylphenol concentration of 10.3 $\mu g \cdot L^{-1}$ (Brooke 1993a). This concentration was multiplied by a safety factor of 0.1 to obtain a water quality guideline for nonylphenol and its ethoxylates of 1.0 $\mu g \cdot L^{-1}$, expressed in NP TEQ units, for the protection of freshwater aquatic life. The toxicity data available for marine aquatic species were sufficient to meet the minimum data requirements for an interim water quality guideline. The critical study was a 28-day test with *Americamysis bahia* which had a LOEC for growth reduction of 6.7 $\mu g \cdot L^{-1}$ (Ward and Boeri 1991a). Multiplying this by a safety factor of 0.1, an interim water quality guideline of 0.7 $\mu g \cdot L^{-1}$, expressed in NP TEQ units, is recommended for the protection of marine (including estuarine) aquatic life. These guidelines, which refer to concentrations in unfiltered samples, are intended to protect all forms of aquatic life and all aspects of aquatic life cycles during an indefinite period of exposure to the water column (CCME 1991). Current limits of detection for nonylphenolic substances in water range from 0.01 $\mu g \cdot L^{-1}$ (for NP) to 1.6 $\mu g \cdot L^{-1}$ (for higher molecular weight NPEs).

For the development of Canadian sediment quality guidelines, there were insufficient data to meet the minimum requirements for any of the three approaches described in the protocol (i.e., National Status and Trends Program Approach (NSTP), Spiked-Sediment Toxicity Test Approach (SSTT), adoption of guidelines from other jurisdictions). Instead, an equilibrium partitioning approach was used to derive provisional interim sediment quality guidelines (ISQGs) from the Canadian water quality guidelines. The revision and re-assessment of these provisional ISQGs will be possible when sufficient data become available to support the use of the modified NSTP or SSTT approaches as described in the formal protocol (CCME 1995). Based on equilibrium partitioning calculations, a provisional interim sediment quality guideline of 1.4 $mg \cdot kg^{-1}$ dry weight, expressed in NP TEQ units, is recommended for the protection of freshwater aquatic life. For the protection of marine (including estuarine) life, a provisional interim sediment quality guideline of 1.0 $mg \cdot kg^{-1}$ dry weight, expressed in NP TEQ units, is recommended. These guidelines are normalized to 1% total organic carbon, but should be adjusted based on local levels of organic carbon in the sediments. These guidelines are intended to be protective of all forms of aquatic life and all aspects of aquatic life cycles during an indefinite period of exposure to bed sediments (CCME 1995). These guidelines refer to total concentrations of nonylphenolic substances in surficial sediments (i.e., top 5 centimetres), as quantified by extraction using standard analytical methods (e.g., soxhlet extraction, steam distillation, supercritical fluid extraction) followed by detection with high pressure liquid chromatography or gas chromatography/mass spectrometry. Current limits of detection for nonylphenolic substances in sediment range from 0.015 to 0.1 $mg \cdot kg^{-1}$ dry weight.

There were sufficient soil toxicity data to meet the minimum requirements for a weight of evidence method for soil contact guideline derivation. However, adequate data were not available for the derivation of a food ingestion guideline value or a nutrient and energy cycling check. For agricultural and residential/parkland land uses, the soil contact guideline was calculated as the 25th percentile of the effects and no effects data distribution, divided by an uncertainty factor of 5. For commercial and industrial land uses, the soil contact guidelines were calculated as the 25th percentile of the effects data distribution only. Therefore, soil quality guidelines for nonylphenol and its ethoxylates for agricultural and residential/parkland land use are both recommended at 5.7 $mg \cdot kg^{-1}$, expressed in NP TEQ units. For both commercial and industrial land uses, soil quality guidelines of 14 $mg \cdot kg^{-1}$, expressed in NP TEQ units, are recommended. These guidelines are optimized for soils within the pH range of 5.8 to 7.5 because the toxicological studies upon which they are based were conducted within this range. The current limit of detection for nonylphenol in soil is 0.05 $mg \cdot kg^{-1}$. A groundwater check of 21 $mg \cdot kg^{-1}$, expressed in

NP TEQ units, was calculated as the concentration of NP and NPEs in soil that should not be exceeded in order to protect freshwater aquatic life from possible groundwater contamination.

Table 1. Summary of Canadian Environmental Quality Guidelines for nonylphenol and its ethoxylates.

Environmental Media	Media Type	Guideline Type	Guideline Value*
Water	Freshwater	Full	1.0 $\mu g \cdot L^{-1}$
	Marine	Interim	0.7 $\mu g \cdot L^{-1}$
Sediment †	Freshwater	Provisional Interim	1.4 $mg \cdot kg^{-1}$
	Marine	Provisional Interim	1.0 $mg \cdot kg^{-1}$
Soil	Agricultural	SQG_E ‡	5.7 $mg \cdot kg^{-1}$
	Residential/Parkland	SQG_E	5.7 $mg \cdot kg^{-1}$
	Commercial	SQG_E	14 $mg \cdot kg^{-1}$
	Industrial	SQG_E	14 $mg \cdot kg^{-1}$
	Groundwater	Check value	21 $mg \cdot kg^{-1}$

* all guidelines are expressed in nonylphenol toxic equivalent units
† these guideline values are for sediments containing 1% total organic carbon
‡ environmental soil quality guideline; a full soil quality guideline would also consider protection for human health (CCME 1996)

RÉSUMÉ

Le présent document d'évaluation scientifique présente les principes qui sont à la base de l'élaboration des Recommandations canadiennes pour la qualité de l'environnement pour le nonylphénol (NP) et les dérivés éthoxylés du nonylphénol (NPE). Il contient une revue de l'information technique de base sur les propriétés chimiques et physiques du NP et des NPE, les sources et les émissions au Canada, la distribution et le comportement des NP et des NPE dans l'environnement et enfin leurs effets toxicologiques sur diverses espèces aquatiques et terrestres. Cette information sert à l'élaboration de recommandations pour la qualité de l'eau, des sédiments et des sols concernant le NP et les NPE, de façon à protéger les récepteurs écologiques dans chacun de ces compartiments de l'environnement. Toutes les Recommandations canadiennes pour la qualité de l'environnement présentées dans le présent document sont basées sur les meilleures données toxicologiques disponibles au moment de sa rédaction. Les recherches de littérature les plus récentes ont été effectuées en août 2000, mais certains renseignements obtenus depuis cette date ont également été inclus suite aux commentaires des examinateurs.

Les NPE sont un groupe de substances chimiques, organiques et non ioniques, utilisées comme tensioactifs, détergents, émulsifiants, agents de mouillage et dispersants dans une vaste gamme de procédés industriels et de produits commerciaux. Ils sont principalement rejetés dans l'environnement via les effluents d'eaux usées provenant des égouts ou les eaux usées industrielles. Les NPE sont soumis à une dégradation primaire initiale impliquant principalement une perte de groupes éthoxy dans les installations de traitements d'eaux usées et à leur entrée dans l'environnement. Des produits de dégradation plus réfractaires, comme le nonylphénol (NP), le nonylphénol monoéthoxylé (NP1EO), le nonylphénol diéthoxylé (NP2EO), l'acide nonylphénoxyacétique (NP1EC) et l'acide nonylphénoxyéthoxyacétique (NP2EC) sont alors formés.

Les substances nonylphénoliques pénètrent l'environnement majoritairement par la voie des rejets d'eaux usées des municipalités et des usines à travers les eaux de surface. Étant donné qu'elles sont employées comme produits de formulation dans certains pesticides, les substances nonylphénoliques peuvent également être déposées sur les eaux de surface suivant l'épandage aérien de pesticides, ou encore, être lessivées à partir de terres agricoles ou forestières traitées par pulvérisation. Dans le milieu aquatique, les substances chimiques les plus hydrophobes, comme le NP, auront tendance à être adsorbées sur les particules et à s'accumuler dans les sédiments de fond. Dans les usines de traitement des eaux usées, une partie du NP sera également distribuée dans les boues et pourra alors devenir un contaminant des systèmes terrestres, lorsque ces boues sont utilisées comme remblai ou appliquées comme amendement du sol sur les terres agricoles. La documentation mentionne le lessivage des substances nonylphénoliques dans les eaux souterraines.

Il a été proposé que le nonylphénol et ses dérivés éthoxylés soient désignés « toxiques » sous la *Loi canadienne sur la protection de l'environnement* (LCPE 1999). De grandes quantités de NPE sont utilisées chaque année, au Canada. Par conséquent, leurs produits de dégradation (c.-à-d. le NP et les NPE à courte chaîne), qui sont persistents, toxiques et qui possèdent un effet oestrogène plus marqués, peuvent être rejetés à des concentrations qui pourraient altérer la vie aquatique et terrestre dans les milieux récepteurs. À la lumière de ces impacts possibles, l'élaboration des Recommandations canadiennes pour la qualité de l'environnement (RCQE) s'est avérée nécessaire. Les RCQE, approuvées à l'échelle nationale et fondées sur des données scientifiques solides, sont des objectifs recommandés sous forme de concentrations qui ne devraient entraîner qu'un risque négligeable pour la santé du biote et de l'écosystème. Ces recommandations ont été élaborées selon les méthodes décrites dans les protocoles approuvés à l'échelle nationale visant l'établissement des Recommandations canadiennes pour la qualité de l'environnement (CCME, 1991; CCME, 1995; CCME, 1996).

Les Recommandations canadiennes pour la qualité de l'environnement élaborées dans le présent document sont résumées dans le tableau 1. Les recommandations figurant dans ce document sont fondées sur les données relatives au nonylphénol, mais, comme décrit au chapitre 3, il est conseillé d'utiliser une approche d'équivalent toxique (QÉT) lors de l'application de ces recommandations à des mélanges de substances nonylphénoliques. En comparant un échantillon de l'environnement aux recommandations, les concentrations de chaque substance nonylphénolique présente dans l'échantillon devraient être multipliées par leur facteur d'équivalence de toxicité (FÉT) respectif, et additionnées pour donner la concentration totale en unités de QÉT de NP. Un ensemble initial de FÉT, fondé sur la toxicité des dérivés éthoxylés et carboxylés du nonylphénol, a été recommandé. D'autres travaux seront nécessaires pour obtenir et valider les valeurs FÉT pour des congénères éthoxylés individuels, mais, d'ici là, il est recommandé d'appliquer les FÉT présentés ici.

Les recommandations pour les résidus dans les tissus du biote aquatique, visant à protéger les espèces fauniques consommant les organismes aquatiques, n'ont pas été déterminées en raison du manque de données de toxicité disponibles pour les mammifères et les oiseaux.

Suffisamment de données de toxicité étaient disponibles pour l'élaboration d'une recommandation complète concernant la qualité de l'eau douce, mais non pour une recommandation concernant la qualité de l'eau marine. La recommandation pour l'eau douce a été basée sur l'effet toxicologique chronique le plus sensible provenant d'une étude primaire de 91 jours, dont la concentration minimale avec effet observé (CMEO) de 10,3 $\mu g \cdot L^{-1}$ (Brooke 1993a) de nonylphénol a entraîné une réduction de la croissance chez la truite arc-en-ciel. Afin de protéger la vie aquatique en eau douce, cette concentration a été multipliée par un facteur de sécurité de 0,1 pour obtenir une recommandation de 1,0 $\mu g \cdot L^{-1}$, exprimée en unités de QÉT de NP, pour la qualité de l'eau vis à vis le nonylphénol et ses dérivés éthoxylés. Les données de toxicité disponibles pour les espèces aquatiques marines étaient suffisantes pour répondre aux exigences de données minimales pour élaborer une recommandation provisoire pour la qualité de l'eau. L'étude critique était un essai de 28 jours avec *Americamysis bahia* démontrant une réduction de croissance associée à une CMEO de 6,7 $\mu g \cdot L^{-1}$ (Ward et Boeri, 1991a). En multipliant cette valeur par un facteur de sécurité de 0,1, une recommandation provisoire pour la qualité de l'eau de 0,7 $\mu g \cdot L^{-1}$ est obtenue, en unités de QÉT de NP, aux fins de la protection de la vie aquatique marine (incluant la vie estuarienne). Ces recommandations, qui correspondent à des concentrations dans des échantillons non filtrés, visent à protéger toutes les formes de vie aquatique et tous les aspects de leurs cycles de vie pendant une période indéfinie d'exposition à la colonne d'eau (CCME, 1991). Les limites de détection actuelles des substances nonylphénoliques dans l'eau se situent dans une plage de 0,01 $\mu g \cdot L^{-1}$ (pour le NP) à 1,6 $\mu g \cdot L^{-1}$ (pour les NPE de masse moléculaire plus élevée).

Aux fins de l'élaboration des recommandations canadiennes pour la qualité des sédiments, les données disponibles étaient insuffisantes pour satisfaire aux exigences minimales de n'importe laquelle des trois démarches décrites dans le protocole (« National Status and Trends Program » (NSTP), tests de toxicité des sédiments avec dopage (TTSD) et adoption de recommandations provenant d'autres juridictions). Une approche de partage à l'équilibre a plutôt été adoptée pour élaborer une version intérimaire des recommandations provisoires pour la qualité des sédiments (RPQS) à partir des recommandations canadiennes pour la qualité de l'eau. La révision et la réévaluation de ces RPQS provisoires seront possibles lorsque les données seront suffisantes pour justifier l'emploi des variantes des méthodes NSTP et TTSD, telles que décrites dans le protocole officiel (CCME 1995). Basée sur des calculs de partage à l'équilibre, une version intérimaire de la RPQS de 1,4 $mg \cdot kg^{-1}$ de poids sec, exprimée en unités de QÉT de NP, fut déterminée pour la protection de la vie aquatique en eau douce. Pour la protection de la vie marine (y compris la vie estuarienne), une version intérimaire de la RPQS de 1,0 $mg \cdot kg^{-1}$ de poids sec, exprimée en unités de QÉT de NP est proposée. Ces recommandations sont normalisées à 1 % de carbone organique total, mais devraient être ajustées en fonction des concentrations locales de carbone organique dans les sédiments. Les recommandations visent à protéger toutes les formes de vie aquatique et tous les aspects de leurs cycles de vie pendant une période

indéfinie d'exposition aux sédiments du lit (CCME, 1995). Ces recommandations correspondent aux concentrations totales de substances nonylphénoliques dans les sédiments de surface (c.-à-d. dans la couche supérieure de 5 cm), mesurées par extraction à l'aide de méthodes analytiques standardisées (p. ex. extraction au soxhlet, distillation à la vapeur, extraction par fluide supercritique), suivie de détection par chromatographie liquide à haute pression ou chromatographie gazeuse/spectrométrie de masse. Les limites de détection actuelles des substances nonylphénoliques dans les sédiments se situent dans une plage de 0,015 à 0,1 $mg \cdot kg^{-1}$ de poids sec.

Suffisamment de données de toxicité étaient disponibles en ce qui a trait au sol pour satisfaire aux exigences minimales d'une méthode fondée sur le poids de la preuve permettant d'élaborer une recommandation pour le contact avec le sol. Par contre, un manque de données ne permettait pas l'obtention d'une valeur recommandée concernant l'ingestion par les aliments ni une vérification du cycle nutritif et énergétique. Dans le cas des utilisations sur les terres à vocation agricole, résidentielle, ou encore destinées aux parcs, la recommandation pour le contact avec le sol a été calculée au 25^{e} percentile de la distribution des données avec et sans effets, valeur par la suite divisée par un facteur d'incertitude de 5. Pour les utilisations sur les terres à vocation commerciale ou industrielle, les recommandations pour le contact avec le sol ont été calculées au 25^{e} percentile de la distribution des données avec effets seulement. Par conséquent, les recommandations pour la qualité du sol dans le cas du nonylphénol et de ses dérivés éthoxylés utilisés sur les terres à vocation agricole, résidentielle, ou encore destinées aux parcs sont toutes deux de 5,7 $mg \cdot kg^{-1}$, en unités de QÉT de NP. Pour les utilisations sur les terres à vocation tant commerciale qu'industrielle, les recommandations pour la qualité du sol sont de 14,2 $mg \cdot kg^{-1}$, en unités de QÉT de NP. Ces recommandations sont optimalisées pour les sols dont le pH se situe entre 5,8 à 7,5, du fait que les études toxicologiques sur lesquelles elles sont fondées ont été effectuées dans cette étendue. La limite de détection actuelle pour le nonylphénol dans le sol est 0,05 $mg \cdot kg^{-1}$. Une valeur de vérification d'eau souterraine de 21,2 $mg \cdot kg^{-1}$, en unités de QÉT de NP, a été calculée comme concentration de NP et de NPE du sol à ne pas dépasser afin de protéger la vie aquatique en eau douce contre une contamination possible par l'eau souterraine.

Tableau 1. Résumé des recommandations canadiennes pour la qualité de l'environnement, concernant le nonylphénol et ses dérivés éthoxylés

Milieu environnemental	Type de milieu	Type de recommandation	Valeur recommandée*
Eau	Eau douce	Complète	1,0 $\mu g \cdot L^{-1}$
	Eau marine	Provisoire	0,7 $\mu g \cdot L^{-1}$
Sédiments †	Eau douce	Provisoire (intérimaire)	1,4 $mg \cdot kg^{-1}$
	Eau marine	Provisoire (intérimaire)	1,0 $mg \cdot kg^{-1}$
Sol	Agricole	RQS_E ‡	5,7 $mg \cdot kg^{-1}$
	Résidentiel/parcs	RQS_E	5,7 $mg \cdot kg^{-1}$
	Commercial	RQS_E	14 $mg \cdot kg^{-1}$
	Industriel	RQS_E	14 $mg \cdot kg^{-1}$
	Eau souterraine	Valeur pour vérification	21 $mg \cdot kg^{-1}$

* toutes les recommandations sont exprimées en unités d'équivalent toxique de NP

† ces recommandations sont valables pour des sédiments renfermant 1% de carbone organique total

‡ recommandation pour la qualité environnementale du sol; une recommandation complète pour la qualité du sol tiendrait également compte de la protection de la santé humaine (CCME, 1996)

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GLOSSARY OF ACRONYMS

AP	alkylphenol
APE	alkylphenol ethoxylate (as a general class)
AP*n*EO	alkylphenol ethoxylate, where *n* = specific number of EO groups
BEDS	Biological Effects Database for Sediments
BOD, COD	biological oxygen demand, chemical oxygen demand
CAS	Chemical Abstracts Service
CCME	Canadian Council of Ministers of the Environment
CEC	cation exchange capacity
CEPA	Canadian Environmental Protection Act
DTED	daily threshold effects dose
DT_{50}, DT_{95}	mean time to 50% dissipation, mean time to 95% dissipation
dw	dry weight
ECL, ERL	effects concentration low, effects range low
EC_{50}, ED_{50}	median effective concentration, median effective dose
EDC	endocrine disrupting chemical
EO	ethoxylate group
EQGs	Environmental Quality Guidelines
GC/MS	gas chromatography/mass spectrometry
HLB	hydrophile-lipophile balance
HPLC	high pressure liquid chromatography
ISQG	interim sediment quality guideline
LC_{50}	median lethal concentration
LOEC, LOEL	lowest observed effect concentration, lowest observed effect level
LOAEL	lowest observed adverse effect level
NE	no effect
NOEC, NOEL	no observed effect concentration, no observed effect level
NP	nonylphenol
NPEC	nonylphenol ethoxycarboxylate or nonylphenoxy acetic acid (as a general class)
NP*n*EC	nonylphenol ethoxycarboxylate, where *n* = specific number of EO groups
NPE	nonylphenol ethoxylate (as a general class)
NP*n*EO	nonylphenol ethoxylate, where *n* = specific number of EO groups
NPER	no potential effects range
NR	not reported
NSTP	National Status and Trends Program
OECD	Organisation for Economic Co-operation and Development
OP	octylphenol
OP*n*EO	octylphenol ethoxylate, where *n* = specific number of EO groups
PEL	probable effect level
PSL	Priority Substance List
QSAR	quantitative structure-activity relationship
SQG_E, SQG_{HH}	environmental soil quality guideline, human health soil quality guideline
SQG_I, SQG_{SC}	soil quality guideline for ingestion, soil quality guideline for soil contact
SSTT	Spiked Sediment Toxicity Test
WWTP	wastewater treatment plant
TEC, TEL	threshold effect concentration, threshold effect level
TEF, TEQ	toxic equivalency factor, toxic equivalency
TOC	total organic carbon
UF	uncertainty factor
USEPA	United States Environmental Protection Agency
WQG	water quality guideline
ww	wet weight

CHAPTER 1. INTRODUCTION

Nonylphenol (NP) is a synthetic substance (typically an isomeric mixture) that belongs to the class of organic chemicals known as alkylphenols (APs), and consists of a nine-carbon alkyl chain attached to a phenol ring (Fig. 1). APs, such as NP, may be used to produce various derivatives, including a class of nonionic surfactants[1] called alkylphenol ethoxylates (APEs). APEs are composed of a phenol group attached to both an alkyl chain [i.e., $-(CH_2)_nH$] and an ethoxylate chain [i.e., $-O(CH_2CH_2O)_nH$]. In the case of nonylphenol ethoxylates (NPEs), the alkyl chain contains nine carbon atoms, and the ethoxylate chain may range in length from 1 to 100 ethoxylate groups.

As nonionic surfactants, NPEs are commonly used in a wide variety of industrial processes, as well as in cleaning products for residential and commercial use. Upon their release to the environment, the major degradation product of NPEs is NP. Therefore, NP is both the parent compound of NPEs during production, and a breakdown product of NPEs upon degradation. There is concern about the occurrence of NP and NPEs in the Canadian environment for a number of reasons. NPEs are released through sewage and industrial effluents in large quantities, and although they undergo a quick initial degradation, their degradation products, including nonylphenol, are much more persistent. NP and NPEs have shown evidence of toxicity to both aquatic and terrestrial organisms, including effects on endocrine systems.

Nonylphenol and its ethoxylates are among the substances on the second Priority Substances List (PSL2) that were required to undergo an environmental risk assessment under the Canadian Environmental Protection Act (CEPA, Bill C-32, June 1, 1999). The draft PSL2 assessment of nonylphenol and its ethoxylates has found that nonylphenol is entering the Canadian environment in quantities or concentrations, or under conditions, that are having, or may have, a harmful effect on the environment or its biological diversity (Government of Canada 2000a). Therefore, it has been proposed that nonylphenol and its ethoxylates be designated as "toxic" according to Section 64 of CEPA. An estimated no-effects value (ENEV) of 1.0 $\mu g \cdot L^{-1}$ was calculated from available water toxicity data for the protection of aquatic biota (Servos et al. 2000). Risk management strategies, that may be developed as a follow-up to the CEPA assessment and the likely designation of NPEs and NP as toxic, will require the use of targets or benchmarks for ensuring sustainability of ecosystem health. Therefore, in complement to the CEPA assessment, this report has been undertaken to develop Canadian Environmental Quality Guidelines (EQGs) to provide such benchmarks for NP and NPEs.

Under the auspices of the Canadian Council of Ministers of the Environment (CCME), Canada has been developing environmental quality guidelines for water, sediment, soil, and tissue quality since the 1980s (CCREM 1987; CCME 1999). Canadian EQGs are nationally endorsed, science-based goals that are recommended as levels that should result in negligible risk to biota and ecosystem health. These guidelines serve as benchmarks or indicators of environmental quality that can be used nationally for the protection, evaluation, and enhancement of the Canadian environment and its beneficial uses. Often the Canadian EQGs form the scientific basis for site-specific criteria, guidelines, objectives, or standards set by the various Canadian provincial and territorial jurisdictions.

This document describes the development of Canadian EQGs for nonylphenol and its ethoxylates for the protection of water, sediment and soil quality. These guidelines are numerical concentrations that are recommended to support and maintain aquatic (both freshwater and marine), sediment-dwelling (both freshwater and marine), and terrestrial life, and are developed

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[1] Nonionic surfactants (as opposed to cationic, anionic, or amphoteric surfactants) contain no ionic constituents and produce electrically neutral colloidal particles in solution (Davidsohn and Milwidsky 1967).

from the available scientific information on the biological effects of nonylphenolic compounds in the environment. Water, sediment and soil have been identified as the key compartments of the environment for which Canadian EQGs should be developed as a result of the potential for impacts on the beneficial uses of the environment from NP and NPEs. Tissue residue guidelines in aquatic biota for the protection of wildlife consumers of aquatic life have not been derived due to the lack of available toxicity data for mammalian and avian species. Nationally approved protocols have been followed in developing Canadian EQGs for the protection of water, sediment and soil quality (CCME 1991; CCME 1995; CCME 1996).

All of the guidelines that have been developed in this document are based on toxicity data for nonylphenol; however, they may be applied to mixtures of nonylphenol, nonylphenol ethoxylates and nonylphenol ethoxycarboxylates (NPECs) with the use of toxic equivalents. In Chapters 3 and 9, guidance is provided on how to account for NPEs and NPECs when implementing the guidelines.

In addition to describing the development of environmental quality guidelines, this document also reviews other available scientific information on nonylphenol and its ethoxylates. This discussion includes a review of the physical and chemical properties of NP and NPEs, sources of these substances to the environment, analytical methods used to detect NP and NPEs, levels of these substances found in the various compartments of the Canadian environment, and their environmental fate and behaviour. Although the EQGs are solely developed using toxicological data, this additional information is discussed in order to provide important considerations for the implementation of the guidelines. More detailed information on some of these aspects has also been summarized and reviewed to support the CEPA PSL2 assessment of NP and NPEs and can be found elsewhere (see Servos et al. 2000).

Physical and Chemical Properties

Nonylphenol is a generic term used to refer to a group of isomeric compounds, each consisting of a nine-carbon alkyl chain ($-C_9H_{19}$) attached to a phenol group, with the molecular formula $C_{15}H_{24}O$. The various isomers can differ both in the degree of alkyl chain branching, and in the position on the phenol ring at which the alkyl chain is attached. Most nonylphenol in production is of the form 4-nonylphenol (i.e., attachment at the *para*-position) with varied alkyl chain branching. Very little straight chain nonylphenol (*n*-nonylphenol) is produced. Commercially produced 4-nonylphenol may also contain small amounts of 2-nonylphenol (i.e., attachment of the nonyl chain to the *ortho*-position of the phenol ring), or 2,4-dinonylphenol (i.e., two nonyl chains attached to a phenol ring) as impurities (European Commission 1999). The Chemical Abstract Service (CAS) registry number for a mixture of various branched isomers of nonylphenol is 84852-15-3 (which may be followed by an asterisk) and is given the IUPAC name of "4-nonylphenol (branched)". Some of the individual branched isomers also have their own specific CAS numbers. Straight chain nonylphenol goes by the IUPAC name of "nonylphenol" and has the CAS number 25154-52-3. NP may also be referred to as "isononylphenol" (CAS number 11066-49-2) or "phenol, nonyl-, branched" (CAS number 90481-04-2) (European Commission 1999). In this document, the term nonylphenol is used to refer to all isomers and is best represented by CAS number 84852-15-3. Data specific to particular isomers generally have not been differentiated.

The major derivatives of nonylphenol are nonylphenol ethoxylates (NPEs) and nonylphenol ethoxycarboxylates (or nonylphenoxy acetic acids, NPECs) (Figure 1). Nonylphenol ethoxylates have the general formula C_9H_{19}-$C_6H_4O(CH_2CH_2O)_nH$, with n ranging from 1 to 100, but with the vast majority of commercially produced NPEs containing between 6 and 12 ethoxylate (EO) groups. Specific NPE oligomers may be reported as NP*n*EO, where *n* refers to the mean number of EO groups in the ethoxylate chain. For example, an oligomer with 9 EO groups is referred to as NP9EO. The term NP9EO may also refer to a mixture of various oligomers for which the mean number of EO groups per molecule is 9 (i.e., the mixture may also contain NP8EO, NP10EO,

etc.). A mixture containing equal proportions of both NP1EO and NP2EO, for example, would be given the abbreviation of NP1.5EO.

Nonylphenol ethoxycarboxylates have the general formula C_9H_{19}-$C_6H_4O(CH_2CH_2O)_nCH_2COOH$. As with NPEs, nonylphenol ethoxycarboxylates with specific ethoxylate chain lengths may be reported as NP*n*EC, where *n* refers to the number of EO groups in the ethoxylate chain.

The basic structures of the three main types of nonylphenolic compounds are shown in Figure 1.

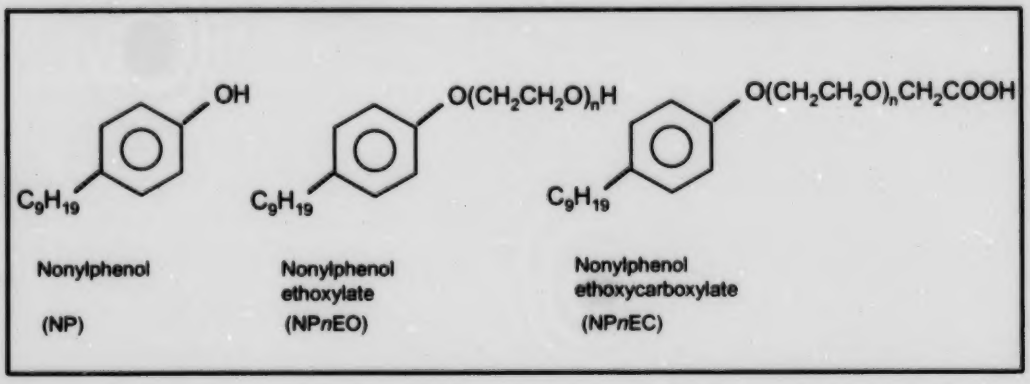


Figure 1. The structure of nonylphenol and its derivatives.

Nonylphenol is a colourless to pale yellow, or straw-coloured, viscous liquid with a slight characteristic phenolic odour (Reed 1978; CCOHS 1995; Budavari et al. 1996). The physical and chemical properties of nonylphenol are listed in Table 2. NP has a molecular weight of 220.3 (Reed 1978). The vapour pressure is 4.55 (± 3.5) x 10^{-3} Pa at 25°C, and Henry's Law Constant is 11.02 Pa $m^3 \cdot mol^{-1}$ (Romano 1991 as cited in Maguire 1999; European Commission 1999). This low Henry's Law Constant suggests that volatilization of nonylphenol from water would not be significant (Maguire 1999). The pK_a (negative logarithm of the acid dissociation constant) of NP is 10.7, indicating that in most natural waters virtually all NP is present in the undissociated form (Romano 1991 as cited in Maguire 1999).

Nonylphenol has a low solubility in water or dilute aqueous NaOH, but is readily soluble in acetone, benzene, chlorinated solvents, aniline, heptane, aliphatic alcohols, and ethylene glycol (Reed 1978; Budavari et al. 1996). Aqueous solubility of nonylphenol (at 20.5°C) is 5.43 $mg \cdot L^{-1}$, with only slight variation of this solubility over the temperature range of 2 to 25°C (Ahel and Giger 1993a). Water solubility is increased by alkyl branching (Talmage 1994). There is also a linear increase in solubility with increasing number of ethoxylate groups. Solubilities (at 20.5°C) for NP1EO, NP2EO, NP3EO, NP4EO, and NP5EO are 3.02, 3.38, 5.88, 7.65, and 9.48 $mg \cdot L^{-1}$, respectively (Ahel and Giger 1993a). NP*n*EOs are water soluble for values of $n \geq 7$ (Talmage 1994). Similar trends were observed with octylphenol (OP) and its ethoxylates (OP*n*EO). Octylphenol ethoxylates have the same chemical structure as NPEs, except that their alkyl chain consists of eight carbons, rather than nine.[1] The solubility of the OP*n*EO oligomers were greater than those of the corresponding NP*n*EO oligomers. Therefore, the lengths of both the ethoxylate and alkyl chains of APEs affect their solubility.

[1] OP and NP also differ with respect to the branching of their alkyl chains. While NP is always present as a mixture of isomers with varied branching of the nine-carbon alkyl chain, OP found in the environment is almost entirely a single isomer with a 1,1,3,3-tetramethylbutyl eight-carbon chain (Swisher 1987; Bennie et al. 1997, 1998).

Nonylphenol ethoxylates have an amphipathic nature, with the alkylphenyl moiety being hydrophobic (i.e., lipophilic) in nature, and the polyethoxylate chain being hydrophilic. This characteristic enables NPEs to concentrate at interfaces between non-mixable phases or liquids (e.g., air and water, oil and water, oil and minerals, biological membranes). Most commercial and industrial applications of nonylphenol ethoxylates utilize this characteristic surfactant property. The sorption and solubilization abilities of a surfactant are related to their hydrophile-lipophile balance (HLB). The higher the molecular weight of the lipophilic component, the more moles of hydrophilic ethoxylate units are needed to make the surfactant water-soluble (Davidsohn and Milwidsky 1967). By calculating the percentage of hydrophilic ethoxylate units compared to the total mass of the surfactant, HLBs for NP5EO, NP8EO, NP9EO, NP14EO, NP17EO, and NP40EO have been estimated at 10, 12.3, 12.8, 14.9, 15.8, and 17.8, respectively (Beigel et al. 1998). When present in solution at high enough concentrations, surfactants will form colloidal-sized clusters, called micelles, in which the hydrophobic tails of the molecules are grouped together in the centre, with the hydrophilic heads pointing out towards the solvent (Haigh 1996). The detergency and solubilisation properties of nonylphenol polyethoxylates are due to this formation of micelles. The concentration at which thermodynamics favour micelle formation is referred to as the critical micelle concentration. Critical micelle concentration values for NP5EO, NP8EO, NP9EO, NP14EO, NP17EO, and NP40EO have been estimated at 13, 32, 42, 66, 112, and 324 $mg \cdot L^{-1}$, respectively (Beigel et al. 1998). Therefore, the more hydrophobic compounds (i.e., those with fewer ethoxylate groups and a lower HLB) are more efficient at micelle formation. Critical micelle concentration values for nonylphenol ethoxylates increase with increasing HLB (Beigel et al. 1998).

Table 2. Physical and chemical properties of nonylphenol and select nonylphenol ethoxylates.

Property	Nonylphenol	NP1EO	NP4EO	NP9EO	NP40EO
CAS registry number	84852-15-3	--	--	--	--
chemical formula	$C_{15}H_{24}O$	$C_{17}H_{28}O_2$	$C_{23}H_{40}O_5$	$C_{33}H_{60}O_{10}$	$C_{95}H_{184}O_{41}$
molecular weight ($g \cdot mol^{-1}$)	220.3	281.4	396.6	616.8	1982.5
freezing point[1] (°C)	<20	--	--	--	--
melting point[1] (°C)	81-83	--	--	--	--
boiling point[2] (°C)	295-320, 310, 304, 293-297	--	--	--	--
flash point[3] (°C)	146; 300 (open cup), 285 (closed cup)	--	--	--	--
density[1] ($g \cdot mL^{-1}$ at 43°C)	0.933	--	--	--	--
specific gravity[4] (at 20°C referred to water at 4°C)	0.95	--	--	--	--
aqueous solubility[5] ($mg \cdot L^{-1}$ at 20.5°C)	5.43	3.02	7.65	water soluble	water soluble
pK_a[6]	10.7	--	--	--	--
log K_{ow}[7]	4.1, 4.2, 4.48, 4.7	4.17, 4.4	4.24, 4.3	4.1	--
vapour pressure[6] (Pa at 25°C)	4.55×10^{-3}	--	--	--	--
Henry's law constant[8] ($Pa\ m^3 \cdot mol^{-1}$)	11.02	--	--	--	--
hydrophile-lipophile balance[9]	--	--	--	12.8	17.8

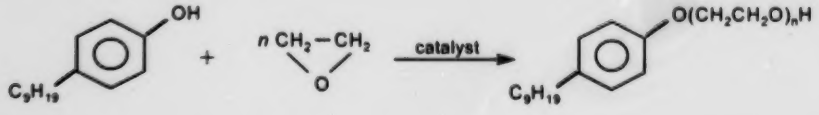
[1] Lorenc et al. 1992
[2] as determined by Reed 1978, Lorenc et al. 1992, CCOHS 1995, and Budavari et al. 1996, respectively
[3] as cited in Lorenc et al. 1992 and Chemical Hazard Response Information System 1995, respectively
[4] Reed 1978
[5] Ahel and Giger 1993a; Hall et al. 1989
[6] Romano 1991 as cited in Maguire 1999
[7] as cited by McLeese et al. 1981, Barber et al. 1988, Freij 1991, Wild and Jones 1992, and Ahel and Giger 1993b (both experimentally determined values and calculated estimations)
[8] European Commission 1999
[9] as cited in Beigel et al. 1998

Production and Uses

The industrial manufacture of nonylphenol involves alkylating phenol with a mixture of isomeric nonenes (propylene trimers) in the presence of an acid catalyst. The resulting nonylphenols (with various isomers of the branched nonyl chain) are recovered by fractional distillation under reduced pressure (Reed 1978). As of 1997, three Canadian companies were producing nonylphenol: Huntsman Canada (Guelph, Ontario), Rhone-Poulenc (Mississauga, Ontario), and Stepan Canada (Longford Mills, Ontario). Total Canadian domestic production of nonylphenol in 1996 (the most recent year for which data are available) was 7700 tonnes, with 5000 tonnes exported (Camford Information Services Inc. 1997). In 1996, a total of 1200 tonnes of NP were imported into Canada, all from the United States. Therefore, the total Canadian domestic demand for nonylphenol in 1996 was 3900 tonnes (Camford Information Services Inc. 1997).

The domestic demand for nonylphenol can be subdivided into its various use applications. In 1989, 1600 tonnes of NP were used to produce ethoxylated pulp mill specialties, 1000 tonnes were needed for ethoxylated textile specialties, 500 tonnes were used to produce miscellaneous ethoxylates, another 500 tonnes were used to produce trinonylphenol phosphite, and 900 tonnes went towards other miscellaneous products (Camford Information Services Inc. 1990). These other miscellaneous products include the use of nonylphenol as an additive in lube oils, and a formulant in pesticides (Camford Information Services Inc. 1990). The most notable Canadian historical example of NP use in pesticides was as a solvent in the Matacil 1.8D formulation of aminocarb. This insecticide, which contained NP at almost three times the weight of the aminocarb, was applied aerially to extensive forested areas of Quebec, New Brunswick, and Newfoundland between 1975 and 1985 to control spruce budworm (Fairchild et al. 1999). Tris(4-nonylphenyl) phosphite is an antioxidant used in the stabilization of rubber, vinyl polymers, polyolefins and styrenics (Lorenc et al. 1992). Nonylphenol may also be used as a catalyst in the curing of epoxy resins, or it may be converted to barium and calcium phenolates which are used as heat stabilizers in poly(vinyl chloride) (Lorenc et al. 1992). As evidenced in the above statistics though, the main use (approximately 70%) of nonylphenol is in the production of nonylphenol ethoxylates.

Nonylphenol ethoxylates are formed through the process of etherification, whereby a basic catalyst is used to condense nonylphenol with ethylene oxide (Reed 1978):



Nonylphenol ethoxylates belong to a class of compounds called alkylphenol ethoxylates (APEs). Of the nonionic surfactants, APEs are the second largest group, after alcohol ethoxylates, in commercial production. Since their introduction in the 1940s, APEs have been widely used in cleaning products and industrial processes (Talmage 1994; White et al. 1994). More than 300 000 tonnes of APEs are produced annually world-wide (Freij 1991; Metcalfe et al. 1996). Nonylphenol ethoxylates account for about 82% of the APE world market, followed by octylphenol ethoxylates (15%) and small amounts of dodecylphenol and dinonylphenol ethoxylates (1-2%; Naylor 1992). In 1990, total use of NPEs in Canada was estimated at 7000 tonnes per year, of which 4100 tonnes of NPEs were produced domestically, while the remainder was imported in cleaning product formulations (Metcalfe et al. 1996). In a 1997 survey of 4500 companies operating in Canada, 189 companies were involved in the production, import, export, shipping, acquisition, use or release of at least 1 tonne per year of NP and/or NPEs (Servos et al. 2000).

Nonylphenol ethoxylates have a broad range of uses due to their effectiveness as detergents, emulsifiers, wetting agents, and dispersing agents. There are eleven major Canadian industrial sectors that use NPEs. These include the pulp and paper industry, textile manufacturing, plastic

and elastomer manufacturing, oil extraction and petroleum production, pesticide formulations, domestic/industrial/institutional cleaners, metal processing, paint and protective coatings, leather manufacturing, building and construction, and the food and beverage sectors. Smaller amounts of nonylphenol ethoxylates are also used in spermicides (Metcalfe et al. 1996).

The commercial and industrial applications of NPEs depend partly upon the length of their ethoxylate chains. Nonylphenol ethoxylates with 4 or 5 ethylene oxide residues per molecule of nonylphenol are typically used as oil-soluble detergents and emulsifiers, and if sulphonated or phosphorylated, can be used as anionic detergents, lubricants and antistatic agents (Reed 1978). NP*n*EOs with 8 to 12 EO groups are the most commonly used compounds; they are utilized in high performance detergents (such as for textile scouring) and in industrial cleaning agents for hard surfaces. Higher ethoxylates (13 to 15 EO groups per molecule), when combined with an oil-soluble anionic surfactant, make excellent emulsifiers for a wide range of solvents and agricultural pesticides (Reed 1978). NP*n*EOs with up to 80 ethoxylate groups are effective as dispersants (Freij 1991).

Directories are available that contain extensive listings of the trade names, manufacturers and distributors of the various NPE products available (Ash and Ash 1997; Anonymous 1999). Examples of some commercial preparations of NPEs include the following products: the Alkasurf CO series manufactured by Rhodia, Inc.; Caloxylate N-9 (NP9EO) manufactured by Pilot Chemical Co.; the Carsonon N series manufactured by Lonza, Inc.; the Cedepal CO series manufactured by Stepan Canada Inc.; the Chemax NP series manufactured by Chemax Inc.; the Delonic NPE series manufactured by DeForest Enterprises, Inc.; the Iconol NP series manufactured by BASF Corp. Performance Chemicals; the Igepal CO series manufactured by GAF Corp. (Rhodia, Inc.); Imbentin-N/7A (NP5EO) and Imbentin-N/200 (NP20EO) manufactured by W. Kolb, AG; Lissapol TN 450 and Lissapol TN XP (both NP8-9EO) manufactured by ICI Chemicals; the Marlophen NP series manufactured by CONDEA Chemie GmbH; the Norfox NP series manufactured by Norman, Fox & Co.; the Polystep F series by Stepan Company; the Surfonic N series manufactured by the Huntsman Corporation; the Tergitol NP series manufactured by Union Carbide Corporation; the TERIC N series manufactured by Huntsman Corp. Australia; and TRITON N by Union Carbide Corporation (Ash and Ash 1997; Anonymous 1999; Lee 1999).

Due to concerns about the toxicity and persistence of NPE degradation products, regulatory and voluntary initiatives have been taken to eliminate many of the uses of NPEs in Europe. In 1986, Switzerland banned the use of NPEs in laundry detergents for domestic use (WWF Canada 1997). In an ordinance enacted in 1991, Swedish parliament decided to phase out 90% of NP and NPE use by the year 2000 (WWF Canada 1997). The Cosmetic, Toiletries, Soap and Detergent Industries Association (SPT) of Denmark have already phased out alkylphenols completely under a voluntary agreement with the Danish EPA (Metcalfe et al. 1996). Germany, Norway, the Netherlands, UK, and France are currently discussing a potential ban and phase out of NPEs in commercial products such as domestic and industrial detergents. In the UK, domestic use of NPEs in detergents has been phased-out since 1976, and some agrochemical companies have voluntarily phased-out NPEs in their pesticides since 1992. In 1995, UK wool scourers volunteered to eliminate discharges (though not use) of APEs by the end of 1996, and several have switched to the use of alcohol ethoxylates (WWF Canada 1997). As of 1990, German wool manufacturers had voluntarily phased out the use of NPEs in wool scouring, and in 1997 a voluntary agreement was drawn up to phase out the use of NPEs in Germany as flocculants in wastewater purification (WWF Canada 1997).

This widespread European phase-out of NPEs is the result of a 1992 recommendation from the Paris Commission. Under Paris Commission Recommendation 92/8, all of the North Sea States and the European Community agreed to phase NPE out of domestic cleaning products by 1995 and out of industrial cleaning products by 2000 (WWF Canada 1997). The Working Group on Diffuse Sources of the Oslo and Paris Conventions for the Prevention of Marine Pollution is currently performing a full risk assessment on nonylphenol (Maguire 1999).

In the United States, nonylphenol was first placed on the Environmental Protection Agency's Inerts List 1 in 1987, identifying it as a pesticide formulant of toxicological concern because of human health and/or ecological considerations (PMRA 2000). Pesticide products in the U.S. which contain nonylphenol must state so on the label. In Canada, the Pest Management Regulatory Agency is proposing regulations to phase out the use of nonylphenol in pesticide formulations by December 31, 2002 (PMRA 2000).

Sources to the Environment

Water

Almost all releases of nonylphenolic compounds are initially routed to the aquatic environment in the form of industrial or municipal wastewater effluents. The bulk of industrial releases to the Canadian environment originate from formulators/distributors of surfactants, and industrial users of cleaning products, degreasers and detergents (Servos et al. 2000). Other significant industrial sources include pulp mills and textile mills, as well as the leather manufacturing, oil extraction, and petroleum production sectors (Metcalfe et al. 1996). Household, industrial, and institutional cleaning products are the major source of the nonylphenolic content in effluents from municipal wastewater treatment plants (WWTPs). Although most nonylphenol and nonylphenol ethoxylates that enter the aquatic environment are introduced via effluent discharge, there is also the potential for these substances to be released to the environment during production, transportation, processing, and during the use of the products (Metcalfe et al. 1996).

Due to their use in some pesticides as adjuvants and dispersants, nonylphenol and nonylphenol ethoxylates may potentially enter the aquatic environment as residues deposited on surface waters from aerial spraying (Ernst et al. 1980; Sundaram et al. 1980; Holmes and Kingsbury 1980; Paveglio et al. 1996) or as runoff from fields. Application of pesticides to forests can introduce NP and NPEs to forest streams and ponds. Application to agricultural fields and orchards is a source of NP and NPEs to drainage ditches and farm ponds, and application to emergent vegetation provides a pathway for entry of NP and NPEs to wetlands and intertidal zones.

Sediment

Once introduced to the aquatic environment, NP and NPEs may adsorb to particulates and be deposited to bottom sediments. Their amphipathic nature enables adsorption to organic matter through hydrophobic reactions as well as adsorption to the mineral components of sediment through hydrophilic reactions (John et al. 2000). Movement of NP and NPEs to the sediments may involve direct adsorption to sediment particles at the surface of the sediment-water interface, or adsorption to suspended sediment particles in the water column that may eventually settle out. Once present in the bottom sediments, there is also the possibility for re-entry of NPEs to the water column through resuspension of sediment, particularly during storms or through dredging.

Soil

The sources of NP and NPEs to the terrestrial environment are much more limited than for the aquatic environment. Some NP or NPEs may enter the soil through the spraying of pesticides on agricultural fields, orchards, golf courses and forests (Sundaram et al. 1980). Another pathway is through the application of sewage sludge or sludge from recycled paper plants to agricultural soils (Diercxsens and Tarradellas 1987; Marcomini et al. 1989a,b; Kirchmann and Tengsved 1991; Hawrelak et al. 1999). Disposal of sewage sludges in landfills may also introduce nonylphenolic compounds into soil (Marcomini et al. 1989a,b; Marcomini et al. 1991).

Despite the strong tendency for adsorption to soil and sediment particles, alkylphenolic compounds (such as NP and NPEs) present in surface waters or landfills may leach into groundwaters (Reinhard et al. 1984; Barber et al. 1988; Zoller et al. 1990; Ahel 1991; Ahel et al. 1996).

Air

Some authors have stated that due to the low volatility of nonylphenolic substances, they are generally not contaminants of the air (e.g., Metcalfe et al. 1996). However, recent studies indicate that in some locations there may be considerable water-to-air volatilization, resulting in high atmospheric concentrations of nonylphenolic substances (Dachs et al. 1999).

The fate processes that control the movement of NP and NPEs through various environmental compartments are discussed further in Chapter 3.

Analytical Methods

Over the past few years there have been major improvements in the methods used for sample extraction and detection of nonylphenol and nonylphenol ethoxylates in environmental samples, both in terms of ease of analysis, and in sensitivity and precision (Kubeck and Naylor 1990). Comprehensive discussions of both historical and more recent analytical methods used for determination of nonylphenolic compounds are provided by Talmage (1994) and Lee (1999). In this document, however, brief descriptions of only the most up-to-date and currently accepted methods are presented. For more detailed information on any of the particular methods that are mentioned below, readers are advised to consult the references given.

Determining concentrations of nonylphenolic compounds in environmental samples involves two main procedures: extraction/clean-up, and detection. The optimal methods to use for extraction and clean-up depend upon the type of environmental sample being analyzed, and the particular oligomer range of interest.

Extraction of nonylphenol and the lower molecular weight nonylphenol ethoxylates (e.g., NP1EO, NP2EO) from aqueous samples can be accomplished through liquid-liquid extraction techniques (Bennie et al. 1997, 1998), or through a combination of steam distillation and solvent extraction by heating the sample to reflux with iso-octane (Kubeck and Naylor 1990) or cyclohexane (Ahel and Giger 1985). With steam distillation methods, no further clean-up of the sample is required before detection analysis. Extraction of higher molecular weight NP*n*EOs requires other methods due to their lower volatility and their higher polarity and solubility. Successful extraction and preconcentration of NPEs, as well as NP and NPECs, from water and effluent samples has been achieved through solid phase extraction (Marcomini et al. 1987, 1990; Kubeck and Naylor 1990; Blackburn and Waldock 1995; Field and Reed 1996; Lee et al. 1998; Lee and Peart 1998; Sekela et al. 1999). Solid phase extraction involves passing the sample through a cartridge or disk containing an adsorbent such as octadecylsilane, graphitized black carbon, or a strong anion exchange resin (Lee et al. 1998). For example, Kubeck and Naylor (1990) used a dual column apparatus in which the aqueous sample was deionized by passing through a mixed-bed ion-exchange resin, then passed through octadecylsilica, which adsorbed the organic material. The organics were flushed from the octadecylsilica with warm methanol, the methanol was removed under a stream of nitrogen, and the remaining residue was redissolved in a dichloromethane/hexane mixture for detection analysis (Kubeck and Naylor 1990).

For extraction of NP, NP1EO and NP2E0 from solid samples (i.e., soil, sediment, sludge, or tissue), Soxhlet extraction (usually with dichloromethane) or steam distillation may be used (Marcomini et al. 1989a, 1989b, 1991; Bennie et al. 1997, 1998). Marcomini et al. (1990) also used a combination of Soxhlet extraction and elution through an aminosilica minicolumn cartridge to extract NP and NPEs from marine sediment and macroalgae. A more recent method using supercritical fluid extraction is a more efficient alternative that may be applied to a wider range of nonylphenolic compounds (Lee and Peart 1995; Lee et al. 1997).

For sensitive detection and quantification, it is now common practice to use high pressure liquid chromatography (HPLC) with fluorescence detection. The phenolic ring in nonylphenolic compounds absorbs ultraviolet light, giving these substances a distinctive fluorescent profile that

can be readily detected (Naylor 1992). With normal-phase HPLC, various nonylphenol ethoxylates can be separated according to their EO chain length on a silica gel matrix using an organic solvent gradient (Talmage 1994). The separated components are then detected by fluorescence and quantitated by comparison with external standards (Talmage 1994). Reversed-phase HPLC may be used to separate different alkylphenol ethoxylates by their alkyl chain (i.e., into nonylphenol, octylphenol, and dodecylphenyl ethoxylates, etc.) (Talmage 1994). Gas chromatography/mass spectrometry (GC/MS) is also widely used for analysis. Extracts containing NP may be analyzed by GC/MS with either electron ionization or chemical ionization (Wahlberg et al. 1990; Clark et al. 1992; Blackburn and Waldock 1995; Bennie et al. 1997; Bennett and Metcalfe 1998). Determination of NPECs is also possible though GC/MS, although they must be derivatized to their volatile esters prior to analysis (Field and Reed 1996). It appears that of the NPEs, only the lower molecular weight compounds can be adequately determined using GC/MS. Wahlberg et al. (1990) were able to quantify NP1EO, NP2EO and NP3EO by first derivatizing them with pentafluorobenzoyl chloride. Clark et al. (1992) found that NPEs with low to moderate degrees of ethoxylation could be determined by GC/MS if present at high concentrations. Clark et al. (1992) also found that NP3-8EO could be detected at the parts per trillion level by using particle-beam liquid chromatography/mass spectrometry.

Although analytical techniques are constantly improving, the analysis of environmental samples for nonylphenolic substances still remains a complicated procedure. Often environmental samples will contain other alkylphenolic surfactants which must be separated from NP and NPEs. Samples may also contain hundreds of different isomers and ethoxamers of NPEs, requiring the use of several different analytical techniques for the detection of all.

Using various combinations of the analytical methods described above, sensitive analyses may be conducted with low limits of detection. The detection limits discussed below were determined through the analysis of environmental samples, and therefore represent concentrations that are environmentally relevant and can be realistically determined from field samples. Reported limits of detection and method detection limits for NP, NP1EO, and NP2EO in water, sewage or effluents are as low as 0.01, 0.02 and 0.02 $\mu g \cdot L^{-1}$, respectively (Bennie et al. 1997, 1998; Rudel et al. 1998). For larger molecular weight NPEs (i.e., NP*n*EOs where n = 3 to 17) method detection limits are higher, at about 1.6 $\mu g \cdot L^{-1}$ (Radian Corporation 1990). Detection limits for individual NPECs in water have been reported between 0.04 and 0.4 $\mu g \cdot L^{-1}$ (Field and Reed 1996). For NP, NP1EO, and NP2EO in sediments, current limits of detection are reported at concentrations as low as 0.004 $mg \cdot kg^{-1}$ (Marcomini et al. 2000). Detection limits for NP in soil have been reported at concentrations as low as 0.05 $mg \cdot kg^{-1}$ (Jobst 1987). For tissue samples, detection limits for NP and each of NP1EO to NP3EO have been reported at 0.001 $mg \cdot kg^{-1}$ and 0.006 $mg \cdot kg^{-1}$, respectively (Wahlberg et al. 1990). With refinements to the standard analytical methods, it may be possible to obtain detection limits lower than those reported here, and in some cases (e.g., Bennie 1998; Sekela et al. 1999) environmental measurements have been noted at concentrations below these detection limits.

CHAPTER 2. CONCENTRATIONS IN CANADA

Nonylphenol and nonylphenol ethoxylates are synthetic compounds that do not occur naturally. Therefore, environmental concentrations can be attributed solely to human activities. A considerable amount of data has been collected on environmental concentrations of nonylphenolic compounds in North America and Europe for fresh water, marine or estuarine water, groundwater, freshwater sediment, and marine or estuarine sediment. Many measurements of these compounds have also been made in sewage effluents, sewage sludge, and industrial effluents. Considerably less data have been collected on soil concentrations of nonylphenolic compounds, and the data that do exist focus on soils that have been amended with sewage sludge.

A comprehensive review and discussion of environmental concentrations of nonylphenol and nonylphenol ethoxylates, both within Canada and outside of Canada, are provided in the CEPA PSL2 assessment of nonylphenol and its ethoxylates (Servos et al. 2000). Therefore, the summary of Canadian environmental concentrations included in this document largely draws upon the above-mentioned assessment. Data were available for NP and NPE concentrations in Canadian fresh surface waters, freshwater and marine sediments, sludge-amended soil, raw sewage, municipal wastewater treatment plant (WWTP) effluent and sludge, and industrial effluents. No data on concentrations in Canadian marine/estuarine waters or groundwater were available.

Surface Waters

Canadian freshwater concentrations of NP and NPEs have been reported for various sites in southern Ontario (largely from rivers, lakes and harbours throughout the Great Lakes - St. Lawrence River system), for freshwater rivers in Quebec, Prince Edward Island, and Nova Scotia, and for sites in the Fraser River of British Columbia. Most reported concentrations of NP fall within the range of <0.01 to 1.7 $\mu g \cdot L^{-1}$ (Bennie et al. 1997; Sekela et al. 1999; Sabik et al. 2000; Rutherford 2001). Agricultural streams monitored in Prince Edward Island in 1999 contained concentrations of NP as high as 0.25 $\mu g \cdot L^{-1}$ (Ernst 2000). The highest measured concentrations occurred in waters near heavily industrialized areas. For example, nonylphenol concentrations directly adjacent to an outfall of a secondary municipal wastewater treatment plant treating textile mill effluent on the Eaton River in Quebec were measured at 94.2 $\mu g \cdot L^{-1}$ (Rutherford 2001). Carey et al. (1981, as cited in Servos et al. 2000) reported NP concentrations as high as 2600 $\mu g \cdot L^{-1}$ in the Canagagigue Creek near Elmira, Ontario; however, these measurements were made following an industrial spill into the small creek, and are not considered representative of typical levels (Servos et al. 2000). NPEs tend to be more ubiquitous than NP, and also occur at higher concentrations. Concentrations of NP1EO and NP2EO have been measured in Canadian fresh waters at <0.02 to 7.8 $\mu g \cdot L^{-1}$, and <0.02 to 16.9 $\mu g \cdot L^{-1}$, respectively (Bennie et al. 1997; Rutherford 2001). Unpublished results from an ongoing survey of 8 rivers in Quebec indicate that the sum of NPE (with 1 to 17 EO groups) ranges between <0.05 and 14.6 $\mu g \cdot L^{-1}$ (Berryman 2000). For the sum of NP1EC and NP2EC, concentrations in Quebec rivers range between <0.05 and 23 $\mu g \cdot L^{-1}$ (Berryman 2000). Levels of NP1EC in water from the St. Lawrence River near Montreal have been reported at 0.014 to 0.51 $\mu g \cdot L^{-1}$, and levels of NP*n*EOs (with *n* ranging from 7 to 16) from the same area have been reported to range from below detection limit to 10.3 $\mu g \cdot L^{-1}$ (Sabik et al. 2000). Extremely high levels of NP and NPEs have been measured in the Annapolis River of Nova Scotia downstream from a textile mill; at 30 metres below the outfall, concentrations of NP, NP1EO, NP2EO, and the sum of NPEs (with 3 to 17 EO groups) were 644, 180, 44.4, and 186 $\mu g \cdot L^{-1}$, respectively (Rutherford 2001). These high levels were attributed to a poorly operating primary treatment system at the mill (Rutherford 2001).

There is currently a lack of data on all nonylphenolic substances for surface fresh waters in Alberta, Manitoba, New Brunswick, Newfoundland, Saskatchewan, and the three territories.

Sediment

Concentration data for nonylphenolics in Canadian freshwater sediments only exist for the Great Lakes - St. Lawrence system, and freshwater locations in British Columbia. Reported freshwater bed sediment concentrations of NP range from below detection limits to 110 $mg \cdot kg^{-1}$ on a dry weight basis (Lee and Peart 1995; Bennie et al. 1997; Brewer et al. 1999; Bennett and Metcalfe 1998, 2000; Sabik et al. 2000). Sekela et al. (1999) reported NP concentrations in suspended solids of the Fraser River, B.C., at 0.021 to 0.087 $mg \cdot kg^{-1}$ dry weight. Canadian freshwater bed sediment concentrations of NP1EO and NP2EO fall within the ranges of <0.015 to 75 $mg \cdot kg^{-1}$ and <0.015 to 18.0 $mg \cdot kg^{-1}$ dry weight, respectively (Bennie et al. 1997; Bennett and Metcalfe 2000; Sabik et al. 2000).

Data on the concentrations of nonylphenolics in Canadian marine sediments are only available for the Strait of Georgia, British Columbia. Concentrations of NP in surficial marine sediments ranged from 0.122 to 0.541 $mg \cdot kg^{-1}$ dry weight (mean = 0.273 $mg \cdot kg^{-1}$, n = 21) (Shang et al. 1999; MacDonald and Ikonomou 2001). Surficial marine sediment concentrations of NP1EO and NP2EO were measured at 0.065 to 0.558 $mg \cdot kg^{-1}$ dry weight (mean = 0.279 $mg \cdot kg^{-1}$, n = 21) and 0.034 to 0.198 $mg \cdot kg^{-1}$ dry weight (mean = 0.069 $mg \cdot kg^{-1}$, n = 21), respectively. Total concentrations of NP*n*EO ($3 \leq n \leq 19$) were measured at 0.29 to 1.42 $mg \cdot kg^{-1}$ dry weight (mean = 0.78 $mg \cdot kg^{-1}$, n = 21). These same researchers also collected marine sediment cores and examined the concentrations of NP and NPEs at various sediment depths. They found that both the total amount of NP*n*EO, and the profile of relative concentrations for individual NP*n*EO ethoxamers did not change significantly with depth (Shang et al. 1999).

Most of the sediments that have been analyzed for NP and NPEs were collected from locations near WWTP outfalls or industrialized areas. Therefore, the data reported here likely represent the upper range of concentrations present in Canadian sediments.

Soil

Only one study has looked at Canadian soil concentrations of nonylphenolic substances, and that study only examined soil from a single site that had been amended with sludge. Water Technology International Corporation (1998a as cited in Servos et al. 2000) measured 2.72 $mg \cdot kg^{-1}$ of NP in sludge-amended soil, <0.538 $mg \cdot kg^{-1}$ each of NP1EC and NP2EC, and only trace amounts of NP1EO, NP2EO, and NP*n*EO.

Sewage and Sludge

Several researchers have reported concentrations of nonylphenolic substances in the raw sewage, primary effluent, final effluent, and sludge of various municipal WWTPs across Canada.

Reported concentrations of NP, NP1EO, NP2EO, NP*n*EO, NP1EC, and NP2EC in raw sewage fall within the ranges of 0.69 to 155 $\mu g \cdot L^{-1}$, 2.8 to 43 $\mu g \cdot L^{-1}$, 0.26 to 38 $\mu g \cdot L^{-1}$, 98.5 to 1300 $\mu g \cdot L^{-1}$, 0.9 to 71.3 $\mu g \cdot L^{-1}$, and 1.4 to 35 $\mu g \cdot L^{-1}$, respectively (Bennie et al. 1998; Lee and Peart 1998; Lee et al. 1998; Water Technology International Corporation 1998b as cited in Servos et al. 2000).

Most studies have found that concentrations of NP and NPEs are lower in the final effluent from municipal WWTPs relative to the incoming raw sewage, with the greatest decreases occurring for the larger NPEs. Reported concentrations of NP, NP1EO, NP2EO, and NP*n*EO in final effluents from Canadian municipal WWTPs are <0.02 to 15.1 $\mu g \cdot L^{-1}$, 0.072 to 26 $\mu g \cdot L^{-1}$, 0.099 to 21 $\mu g \cdot L^{-1}$, and from below detection to 13.3 $\mu g \cdot L^{-1}$, respectively (Lee and Peart 1995; Bennie et al. 1998; Lee and Peart 1998; Sekela et al. 1999; Water Technology International Corporation 1998b as cited in Servos et al. 2000). Rogers et al. (1986) also identified NP, NP1EO and NP2EO in municipal wastewater effluents from Vancouver, B.C.; however, they did not report concentrations.

Sewage treatment appears to have the opposite effect on NPECs, with higher concentrations occurring in the final effluent relative to the raw sewage. Concentrations of NP1EC and NP2EC in

final effluents from Canadian municipal WWTPs have been reported at 1.9 to 703 $\mu g \cdot L^{-1}$ and 11 to 565 $\mu g \cdot L^{-1}$, respectively (Lee and Peart 1998; Lee et al. 1998; Water Technology International Corporation 1998b as cited in Servos et al. 2000). Similar observations of elevated levels of NPECs following secondary treatment have also been observed in several Swiss municipal WWTPs (Ahel et al. 1987). NP1EC and NP2EC are biodegradation products of NPEs, which explains their occurrence at higher concentrations in sewage following treatment (Ahel et al. 1987).

Substances that are more hydrophobic, such as NP and the lower NPEs, have a greater tendency to adsorb to particles and thus accumulate in the sludge during treatment. Concentrations of NP, NP1EO, and NP2EO in the sludge of Canadian municipal WWTPs have been reported at 8.4 to 850 $mg \cdot kg^{-1}$, 3.9 to 437 $mg \cdot kg^{-1}$, and 1.5 to 297 $mg \cdot kg^{-1}$, respectively (Lee and Peart 1995; Lee et al. 1997; Bennie et al. 1998; Water Technology International Corporation 1998b as cited in Servos et al. 2000). More hydrophilic substances, such as the NPECs and the higher NPEs, occur at lower concentrations in sludge. Reported sludge concentrations for NP*n*EO, NP1EC and NP2EC are 9 to 169 $mg \cdot kg^{-1}$, <0.5 to 25 $mg \cdot kg^{-1}$, and <0.5 to 38 $mg \cdot kg^{-1}$, respectively (Lee et al. 1997; Water Technology International Corporation 1998a,b as cited in Servos et al. 2000).

The concentrations and relative proportions of various NPEs in final effluents and sludges vary considerably among municipal WWTPs and reflect differences in the type of effluent and sludge treatment used. The operating efficiencies of individual WWTPs may also affect the levels of NPEs in final effluent and sludge (Ahel et al. 1994a). Differences in NP and NPE concentrations among the raw sewages entering municipal WWTPs are likely a function of several factors including the size of the population served by the WWTP, and the number and types of commercial or industrial clients contributing to the WWTP.

Industrial Effluents and Sludge

Little data exist for concentrations of nonylphenolic compounds in Canadian industrial effluents and sludge, but most of the data that do exist are from pulp and paper mills or textile mills.

Pulp mill effluents have been found to contain NP, NP1EO and NP2EO in concentrations of <0.02 to 26.2 $\mu g \cdot L^{-1}$, <0.015 to 25.9 $\mu g \cdot L^{-1}$, and <0.015 to 35.9 $\mu g \cdot L^{-1}$, respectively (Bennie 1998 as cited in Servos et al. 2000). Unbleached whitewater from a Canadian pulp mill was also reported to contain NP*n*EO at a concentration of 310 $\mu g \cdot L^{-1}$ (Sithole and Allen 1989). Lee and Peart (1999) measured concentrations of nonylphenolics in both the effluent and sludge of 19 Canadian (and 1 U.S.) pulp and paper mills that use a variety of process types and waste treatment methods. Secondary treated effluent contained NP, total NPEs, NP1EC, and NP2EC of <0.1 to 4.32 $\mu g \cdot L^{-1}$, <2 to 71.3 $\mu g \cdot L^{-1}$, <1.0 to 10.13 $\mu g \cdot L^{-1}$, and <1.0 to 32.32 $\mu g \cdot L^{-1}$, respectively (Lee and Peart 1999). Effluents commonly contained NPE oligomers with 10 or fewer ethoxy groups, but ethoxylates with greater than 10 ethoxy groups were seldom detected. Sludge samples from the mills contained NP, total NPEs, NP1EC, and NP2EC at concentrations of <0.05 to 120.9 $mg \cdot kg^{-1}$, <1 to 90.8 $mg \cdot kg^{-1}$, <1 to 5.78 $mg \cdot kg^{-1}$, and <1 to 18.5 $mg \cdot kg^{-1}$ dry weight, respectively (Lee and Peart 1999). Generally, the NPE oligomers that occurred at the highest concentrations in the sludge were NP8EO and NP9EO. Sludge from a Canadian recycled paper plant that was used as an agricultural soil amendment was found to contain NP, NP1EO and NP2EO at concentrations as high as 4.61, 1.21, and 0.39 $mg \cdot kg^{-1}$ dry weight, respectively (Hawrelak et al. 1999).

Textile mill effluents appear to contain considerably greater levels of nonylphenolic substances than pulp and paper mills, with concentrations of NP, NP1EO, NP2EO, and NP*n*EO (n=3 to 17) reported at 1.1 to 13.3 $\mu g \cdot L^{-1}$, 0.74 to 260 $\mu g \cdot L^{-1}$, 0.64 to 590 $\mu g \cdot L^{-1}$, and 50.2 to 8600 $\mu g \cdot L^{-1}$, respectively (Bennie 1998 as cited in Servos et al. 2000).

Analysis of two oil refinery effluent samples did not reveal any detectable concentration of NP (Bennie 1998 as cited in Servos et al. 2000).

CHAPTER 3. ENVIRONMENTAL FATE AND BEHAVIOUR

The occurrence of nonylphenol and its ethoxylates in aquatic or terrestrial ecosystems poses a potential risk to exposed organisms. The bioavailability and toxicity of nonylphenolic compounds are dependent on a number of physical and chemical processes that affect the fate and behaviour of these compounds in the environment. Therefore, understanding the role of these factors is essential for interpreting toxicity data, developing and using environmental quality guidelines, and implementing site-specific objectives. This chapter provides a discussion of the factors that govern the fate and behaviour of nonylphenolic compounds in the environment. The major processes affecting the persistence, distribution and bioavailability of nonylphenolic compounds in the environment include biodegradation, adsorption to suspended solids with subsequent sedimentation, soil particle adsorption, infiltration to groundwater, volatilization, and photolysis. Aerobic and anaerobic biodegradation of nonylphenolic substances by microorganisms is the most important factor affecting their fate. Various other biotic interactions are also involved in the fate and behaviour of nonylphenolic compounds. Uptake and bioaccumulation of nonylphenolic compounds by higher organisms may occur, as well as biotransformation, metabolism, and excretion. Nonylphenolic compounds, if available to biota at sufficiently high concentrations, have the potential to cause toxicity and/or endocrine disruption.

Physical and Chemical Processes

The most important physicochemical process affecting nonylphenolic substances is particle adsorption. Abiotic degradation is not thought to contribute significantly to the dissipation of nonylphenolic substances. Laboratory studies have shown that under aseptic conditions nonylphenol is very stable (Sundaram and Szeto 1981; Corti et al. 1995). Therefore, the effects due to physicochemical transformations such as hydrolysis and photolysis are thought to be negligible (Trocmé et al. 1988; European Commission 1999). Volatilization from either water or soil is also considered an insignificant fate process for nonylphenolic substances (European Commission 1999). Nonetheless, these processes may occur to a minor degree, and therefore are discussed below.

Water

Most of the nonylphenol and nonylphenol ethoxylates entering the environment are initially released to surface waters. Parent nonylphenol polyethoxylates do not appear to be persistent in natural waters, while some breakdown products (e.g., NP, NP1EO, and NP2EO) are moderately persistent (Maguire 1999). The mean time to 50% dissipation (DT_{50}) of 4-NP from the water column was estimated in a mesocosm study to be 0.74 days, and mean time to 95% dissipation (DT_{95}) was 13.8 days (Heinis et al. 1999). In another study where nonylphenol was aerially applied to a forested plot containing a stream, nonylphenol residues were found to persist in the stream water for no more than five days, and a half-life of 2.5 hours was estimated (Sundaram et al. 1980). While in the aquatic environment, potential abiotic fate processes for nonylphenolic compounds include photolysis, volatilization and adsorption to suspended solids.

Photolysis, the decomposition of chemicals by the action of radiant energy, is one process by which nonylphenol and its ethoxylates may be degraded. This process is most effective in the surface layers of the water column where compounds are exposed to sunlight at higher intensities. The first order rate constant for sunlight photolysis of nonylphenol in fresh water has been estimated to range from 0.05 to 0.09 $m^2kW^{-1}h^{-1}$ (Ahel et al. 1994d). In other words, if exposed to continuous noon, summer sunlight, NP in the surface layer of natural waters would have a half-life of 10-15 hours (Ahel et al. 1994d). In deeper water layers this half-life would be considerably longer (e.g., at a depth of 20-25 cm the photolysis rate would be 1.5 times slower than at the surface). The photochemical oxidation of nonylphenol ethoxylates is significantly slower than that of NP (Ahel et al. 1994d). Often the photolytic process is aided by the presence of metals or metallic oxides that can act as photocatalysts. Degradation of alkylphenol ethoxylates is known to be induced by the catalysts Fe (III) and titania ($Ti O_2$) in the presence of

sunlight. The degradation process involves a hydroxyl radical attack on the ethoxylate chain, and, at a much slower rate, on the benzene ring (Pelizzetti et al. 1989; Brand et al. 1998). Further degradation involves oxidation to lower molecular weight products such as acidic compounds, polyethylene glycols, ethylene glycols, and ultimately to carbon dioxide (CO_2).

Variable results have been found for the effect of temperature on the rate of nonylphenol degradation in water. Ahel et al. (1994b) found that concentrations of nonylphenolic compounds in fresh water tend to decrease with higher temperatures. Schöberl and Mann (1976) found little effect of temperature on degradation of NP9EO in fresh water, with 33-36% decomposition occurring at 20-23°C, and 37% at 3-4°C. In contrast, they found a substantial temperature effect in seawater, with 95% degradation occurring at 20-23°C, and only 15% at 3-4°C. Manzano et al. (1999) observed significant increases in the rates of both primary biodegradation and mineralization with increased temperature. They also observed shorter acclimation periods at higher temperatures. Tanghe et al. (1998) found that degradation of nonylphenol in lab-scale activated sludge units was almost complete at 28°C, but slowed down to 13-86% elimination capacity at temperatures of 10-15°C, a finding that is significant considering Canadian conditions. Marcomini et al. (2000) observed considerable seasonality in the concentrations of nonylphenol ethoxylates present in the lagoon of Venice, Italy, and attributed this to the dependence of their biodegradation on temperature.

One potential mechanism by which organic compounds may leave surface waters is through volatilization. In a recent study, nonylphenol was measured in the atmosphere over the Hudson River Estuary at concentrations ranging from 0.1 to 68.6 $ng \cdot m^{-3}$ (Dachs et al. 1999). The authors concluded from these high concentrations that a considerable amount of water-to-air volatilization must have occurred. These results are surprising because most of the previous literature has claimed that volatilization of nonylphenol is not likely to occur under most conditions. The Henry's Law Constant for nonylphenol, which describes the likelihood for a chemical to move from surface water to the air, is 11.02 $Pa\ m^3 \cdot mol^{-1}$ (Reed 1978; European Commission 1999). This low Henry's Law Constant indicates that volatilization is not likely to be a significant mechanism for removal of nonylphenol from water systems and that it is unlikely to be transported very far in the atmosphere (Maguire 1999; European Commission 1999).

Movement of dissolved compounds from the aqueous phase of the water column to the particulate phase may occur through adsorption. Studies have shown that nonylphenol in water is readily adsorbed onto suspended solids (Kvestak et al. 1994; Sekela et al. 1999). Using field data from the Fraser River in British Columbia, the tendency for nonylphenol to partition from the aqueous phase to the suspended solid phase has been evaluated based on organic carbon (OC) content. The sorption partition coefficient (log K_{OC}) for nonylphenol was estimated to be in the range of 4.7 to 5.6 (Sekela et al. 1999). Partitioning of nonylphenol polyethoxylates between the dissolved and particulate phases can vary slightly among various oligomers; NP6EO and NP7EO have shown higher relative tendencies to partition into the particulate phase than any other NPEs (Kvestak et al. 1994).

Sediment

NP and NPEs adsorbed to suspended particles may settle out of the water column into the sediments. Nonylphenolic compounds dissolved in the water may also directly adsorb to particles on the sediment surface. John et al. (2000) suggested that the adsorption of nonylphenol polyethoxylates to sediment involves a combination of two main processes: hydrophobic interactions with organic matter in the sediment, and hydrophilic interactions with the mineral components. These two processes have opposing effects in terms of the ethoxylate chain length. The hydrophobic interactions increase with decreasing ethoxylate chain length since the lower ethoxamers have higher octanol-water partition coefficients (see Table 2) and are more hydrophobic. The hydrophilic interaction involves hydrogen-binding due to the hydration of metal oxides in the sediment to the ether oxygens of the ethoxylate chain. Therefore, the amount of adsorption due to hydrophilic interactions increases with increasing ethoxylate chain length. Partitioning of nonylphenol ethoxylates between the water and sediment phases, and

achievement of adsorption equilibrium occurred rapidly under artificial conditions (John et al. 2000). In a littoral enclosure study, some of the nonylphenol applied through subsurface injection into the water column was found to adsorb to the sediment within two days of the initial application, as well as adsorbing to the plastic walls of the mesocosm enclosures (Heinis et al. 1999). Nonylphenol also appears to be persistent in sediments; in the same freshwater mesocosm study, the DT_{50} of nonylphenol from sediments was estimated at 66.0 days, and the DT_{95} was estimated at 401 days (Heinis et al. 1999). This indicates that there is the potential for carry-over from one year to the next, such that a site which receives regular inputs will have increasing sediment concentrations of NP over time. More studies are needed though to confirm the fate of nonylphenol in freshwater sediments under natural conditions.

Persistence has also been observed in marine sediments. NPEs (with 1 to 14 ethoxylate units) applied to estuarine intertidal plots as part of a herbicide formulation showed decreasing concentrations in the sediments during the first 14 days post-treatment, but then stabilized and remained constant even after 119 days (Paveglio et al. 1996). The authors suggest that this stabilization may have been due to partitioning of the NPEs to sediment with reduced availability for biodegradation; also, lower temperatures at the later dates may have resulted in reduced microbial degradation.

Soil

The abiotic fate processes affecting nonylphenol and its ethoxylates in soils, in decreasing order of importance, include particle adsorption, infiltration to groundwater, and volatilization.

The major route for nonylphenolic compounds to enter terrestrial ecosystems is through the application of sewage sludge or pulp and paper mill sludge to agricultural soils, or through disposal of sludge in landfills. There appear to be conflicting reports on the persistence of nonylphenolic compounds in soil. Some researchers report that under aerobic conditions, nonylphenolic compounds are not persistent and degradation occurs readily (Wild and Jones 1992; Maguire 1999). For example, concentrations of nonylphenol introduced into agricultural soils through sludge application decreased by 90% in ten days (Kirchmann et al. 1991) and by 92% in three months (Diercxsens and Tarradellas 1987). In contrast, there is also evidence of persistence with complete degradation taking considerably longer. In a Swiss study where digested sewage sludge was applied to an experimental field plot, soil concentrations of NP, NP1EO and NP2EO decreased rapidly during the first 10 days after application, with half-lives of 7 to 8 days for each compound (Marcomini et al. 1988). This was followed by a transition period of about 90 days, during which the compounds disappeared at a much slower rate; the half-lives for NP, NP1EO and NP2EO were 90, 150, and 110 days, respectively. The authors continued to measure the concentrations of the nonylphenolic compounds until 330 days after the sewage application, and for the rest of that period, the concentrations remained relatively constant, with estimated half-lives of >360 days. Thus, a small fraction of the initial NP, NP1EO and NP2EO was highly persistent, possibly because its strong sorption to the soil made it unavailable for biodegradation. It is unknown whether this constant residual concentration of NP simply represents the threshold for biological transformation, and therefore will remain the same, or whether the soil is at risk of increasing accumulation of NP with further sludge applications (Marcomini et al. 1988, 1989b). Topp and Starratt (2000) also found that NP underwent a biphasic mineralization in agricultural soil. During a rapid initial phase, that lasted 10 days, about 30% of the nonylphenol (radioactively-labelled at one of the phenolic ring carbons) was converted to carbon dioxide; this was followed by a much slower second phase. Under anaerobic conditions, such as in landfills, it appears that nonylphenolic compounds are persistent in soil (Maguire 1999). For example, in a survey of several German sludge-only landfills, concentrations of NP and NP1EO of 4 and 19 $mg \cdot kg^{-1}$ dry weight, respectively, were detected in samples of sludge that had been landfilled 30 years previously (Marcomini et al. 1989b).

Based on physical/chemical properties, Wild and Jones (1992) speculated that nonylphenol in sludge applied to agricultural soils would likely have high adsorption to soil particles. Various NPEs have also exhibited high adsorption on soil (Beigel et al. 1998). The relative degree of

adsorption among NPE oligomers appears to increase with decreasing number of ethoxylate units, to a maximum soil affinity at about NP9EO, after which the tendency for adsorption decreases with decreasing number of ethoxylate units (Beigel et al. 1998). It is suggested that adsorption increases due to increasing hydrophobicity, but with the shorter ethoxylate chains, that have a lower critical micelle concentration, there may be a preference for surfactant-surfactant interactions and micelle formation, rather than surfactant-soil surface interactions (Beigel et al. 1998).

Although the high adsorption of nonylphenolic compounds to soil particles suggests that there would be a low probability of leaching (Wild and Jones 1992), numerous studies have shown evidence of infiltration of nonylphenolic compounds into groundwater from municipal landfills (Ahel 1991), from contaminated rivers and wastewater canals (Zoller et al. 1990; Ahel 1991; Ahel et al. 1996), and due to sewage disposal through rapid infiltration (Barber et al. 1988; Rudel et al. 1998). Due to their higher water solubility and mobility, nonylphenol ethoxycarboxylates (i.e., NP1EC and NP2EC) are thought to pose a greater risk as groundwater contaminants than other nonylphenolic compounds (Ahel et al. 1996). Although NP, NP1EO and NP2EO are more effectively retained in the soil, significant concentrations of these substances have been measured in groundwater at distances greater than 100 metres from the original source (Ahel 1991; Ahel et al. 1996).

As in water, nonylphenol is not likely to volatilize from soils in significant quantities either. For example, of the initial 1000 $mg \cdot kg^{-1}$ nonylphenol that was spiked into a compost-sandstone mixture, only 0.22% was lost through volatilization over a period of 40 days (Trocmé et al. 1988).

There is currently no information available in the literature on photolysis of nonylphenolic substances on soils.

Biodegradation

The most important mechanism determining the fate of nonylphenolic compounds in water, sediment, or soil, is likely biological degradation and transformation (Ahel et al. 1994b). NPEs undergo a two-stage biodegradation process (refer to Figure 2). **Primary biodegradation** is the process by which the molecules of NPE are oxidized or altered sufficiently to remove their characteristic detergent properties (i.e., surfactancy, formation of foam). Often this results in the formation of intermediate biodegradation products such as mono- (NP1EO) and di-ethoxylates (NP2EO), nonylphenoxy acetic acid (NP1EC), nonylphenoxy ethoxy acetic acid (NP2EC), and NP. **Ultimate biodegradation** (or total biodegradation) refers to the complete conversion of the molecules to CO_2, H_2O, and inorganic salts (Swisher 1987). In sewage treatment processes, efficient elimination of nonylphenol ethoxylates through primary biodegradation may occur, however, ultimate biodegradation is limited due to the formation of products that are biorefractory, i.e., that resist further biodegradation (Ahel et al. 1994a; Maguire 1999).

Primary Biodegradation

Nonylphenol ethoxylates undergo a relatively slow degradation process compared with some other types of surfactants. Steric effects due to the aryl group prevent the occurrence of central ether fission, and a branched alkyl chain reduces the likelihood of ω-/ß-oxidation[1] (White 1993). The major route of biodegradation is most likely to be ethoxylate shortening through the stepwise removal of ethoxy groups (White 1993; Jones and Westmoreland 1998; Maguire 1999). Therefore, the intermediate degradation products of primary biodegradation are lower NPE ethoxamers, or NPECs (depending on environmental conditions), and nonylphenol. Under anaerobic conditions (as in the anaerobic digestion of sludge in sewage treatment plants) the

[1] ω-/ß-oxidation is a two-step process which results in the shortening of an alkyl chain. Through ω-oxidation, the terminal unsubstituted carbon of the alkyl chain is oxidized to form a carboxylic acid (White 1993). With ß-oxidation, the carboxylic acid is further oxidized at the ß-carbon atom, resulting in the removal of a 2-carbon fragment (Manahan 1994).

intermediate breakdown products include NP1EO and NP2EO; under aerobic conditions (that would be found in most natural river waters and wastewater effluents) major degradation products include NP1EC and NP2EC (Ahel et al. 1994b; Maki et al. 1996). Nonylphenol is a degradation product under both aerobic and anaerobic conditions, although formation of NP appears to be more likely under anaerobic conditions (Giger et al. 1984). Degradation of the hydrophobic moiety (i.e., the alkyl chain and phenol group) of NPEs has also been observed, but, it occurs at only half the degradation rate of the hydrophilic polyethoxylate chain (Jones and Westmoreland 1998).

Biodegradabilities of both NP1.5EO and NP9EO have been assessed using the CO_2 Evolution Modified Sturm Test, as described in the Organisation for Economic Co-operation and Development (OECD) guideline 301B (Gledhill, 1999). According to the results from these tests, although the substances are both inherently biodegradable (with >50% mineralization occurring over the 35-day test period), neither meets the requirements for classification as "readily biodegradable" (i.e., ≥ 60% CO_2 produced within 28 days, with this value being achieved within a 10-day window). Among the higher molecular weight NPEs (i.e., with 8 to 30 EO groups) individual ethoxamers do not appear to differ significantly in their biodegradabilities (Rudling and Solyom 1974). Sulphated nonylphenol ethoxylates are less readily biodegraded than non-sulphated NPEs (Pitter and Fuka 1979).

Studies examining biodegradation efficiencies in wastewater treatment plants (WWTPs) using activated sludge have reported greater than 90% primary degradation of NPEs (Rudling and Solyom 1974; Lee and Peart 1998). Lee and Peart (1998) examined biodegradation in a southern Ontario municipal WWTP using secondary treatment with activated sludge. Through aerobic biodegradation, concentrations of NPEs and NP entering the WWTP in raw sewage were reduced by more than 90 and 82%, respectively, in the final effluent. The degradation of NPEs resulted in the formation of carboxylates, as evidenced by 418 and 468% increases in concentrations of NP1EC and NP2EC, respectively, from the raw influent to the effluent. The median total alkylphenolic concentrations were reduced by 53%. The authors speculated that much of the "eliminated" nonylphenolics may have been bound to the sludge, that was used for landfill applications, thus making the total environmental loading even higher. It has been estimated that at least 63% of the total molar mass of all nonylphenolic compounds entering WWTPs is released to the environment; 25% as NP, 19% as NPECs, 11% as lipophilic nonylphenol ethoxylates (NP1EO + NP2EO), and 8% as higher NPEs (Ahel et al. 1994a). The majority of these compounds are released through the secondary effluents, except for NP which is primarily disposed via the digested sewage sludge (Ahel et al. 1994a; Brunner et al. 1988).

Degradation of NPEs has also been observed during the composting of sludges from wool scour effluents (Jones and Westmoreland 1998). Over a 14 week period, NPE concentrations were observed to decrease by more than 96%.

Laboratory studies have been conducted to simulate biodegradation processes that would occur in natural waters. For example, Potter et al. (1999) examined the static die-away of NP18EO added to estuarine water collected from Tampa Bay, Florida. They observed a lag-period of 0 to 12 days, followed by a rapid primary degradation, with primary degradation complete within 4 to 24 days. The first major degradation product to form was NP2EO, which then decayed to NP2EC, a more persistent degradation product. No formation of NP was observed, perhaps because the test conditions were aerobic. Ahel et al. (1994c) also conducted die-away tests by adding NP1EO and NP2EO to river water containing pre-exposed bacterial cultures. They found that biotransformation rates were 1.5 times faster when the water was stirred, than when it remained static. Biotransformation rates were also faster at higher temperatures, with 50-60%

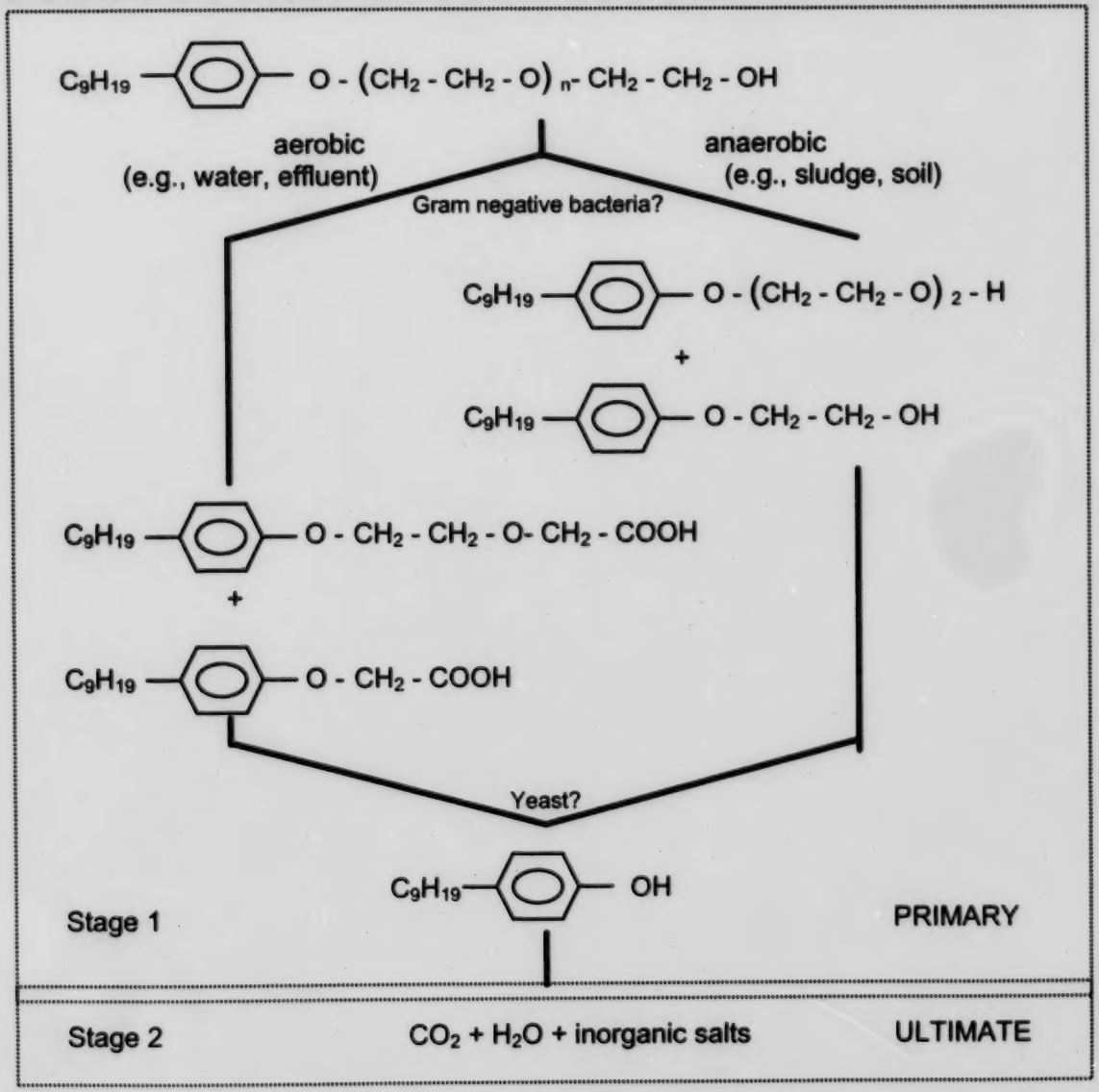

C_9H_{19} — O - $(CH_2 - CH_2 - O)_n$ - $CH_2 - CH_2 - OH$
Nonylphenol polyethoxylate (NPnEO; n = 0 to 99)
aerobic (e.g., water, effluent)
anaerobic (e.g., sludge, soil)
Gram negative bacteria?
C_9H_{19} — O - $(CH_2 - CH_2 - O)_2$ - H
Nonylphenol diethoxylate (NP2EO)
+
C_9H_{19} — O - $CH_2 - CH_2 - OH$
Nonylphenol monoethoxylate (NP1EO)
C_9H_{19} — O - $CH_2 - CH_2 - O - CH_2 - COOH$
Nonylphenoxy ethoxy acetic acid (NP2EC)
+
C_9H_{19} — O - $CH_2 - COOH$
Nonylphenoxy acetic acid (NP1EC)
Yeast?
C_9H_{19} — OH
Nonylphenol (NP)
Stage 1
PRIMARY
Stage 2
$CO_2 + H_2O$ + inorganic salts
ULTIMATE
Carbon dioxide, water, and inorganic salts

Figure 2. Pathways for biodegradation of nonylphenol polyethoxylates.

transformation after 12 days at 20°C compared with 10-20% at 4°C. A control sterile sample showed no evidence of analyte transformation throughout the entire study (Ahel et al. 1994c).

In a few studies, specific microorganisms capable of biodegrading nonylphenolic compounds have been identified. A strain of the bacteria *Pseudomonas* sp. isolated from activated sludge of a municipal WWTP was observed to degrade the EO chain of nonylphenol polyethoxylates to form NP2EO and NP2EC (Maki et al. 1994). Frassinetti et al. (1996) also isolated two strains of *Pseudomonas* sp. and a strain of *Xanthomonas* sp. capable of degrading NP9EO, however none of these were able to assimilate NP. Biotransformation of nonylphenol has been observed with a strain of the yeast *Candida maltosa*, isolated from the sludge of a plant treating textile industry wastewater (Corti et al. 1995). The major degradation product was 4-acetylphenol, and the authors suggest ß-oxidation, followed by decarboxylation, as a possible biodegradation process. It should be noted, however, that the study by Corti et al. (1995) used straight-chain NP; with branched-chain isomers of NP, it is likely that ß-oxidation would occur much less readily (see White 1993).

Microorganisms are more efficient at the biodegradation of nonylphenolic compounds if they have been previously exposed and given time for adaptation; however, pre-exposure is not necessary for biodegradation to occur. For example, biotransformation of NP1EO and NP2EO in synthetic sewage using aerobic bacterial cultures was examined by Ahel et al. (1994c). The bacterial cultures were isolated from three different locations: the wastewater of a detergent manufacturing plant, from the water of a polluted river, and from a pristine forest soil in Croatia. Biotransformation occurred quite rapidly with both the river water and wastewater cultures, while biotransformation with the forest soil culture was less efficient.

Ultimate Biodegradation.

Nonylphenol, NP1EO, NP2EO, NP1EC, and NP2EC are very refractory compounds that are much less readily degraded than their parent compounds, the nonylphenol polyethoxylates. Partial biodegradation and biotransformation of these compounds will occur, but as evidenced below, there are fewer reports of complete degradation (i.e., to CO_2, H_2O, and inorganic salts) than there are of primary degradation of NPEs.

Ekelund et al. (1993) examined the biodegradation of 4-nonylphenol in seawater, both with and without sediment present, through the use of radioactive labelling. Initial degradation in the seawater alone was very slow, with about 0.06% of the NP degraded per day, but after four weeks this rate had increased to 1% per day. The authors suggested that this increase was due to adaptation of the microorganisms to the substrate. In the presence of sediment, the degradation rate was much quicker, at 1.2% per day from the onset, and remained relatively constant. The faster rate with sediment can be accounted for by the larger number of microorganisms that would be available, thus increasing the probability of bacteria able to degrade the NP being present. This rate did not increase with adaptation (as it did in the water only treatment) because adsorption to sediment particles likely reduced NP bioavailability. In the presence of sediment, but under anoxic conditions, the biodegradation rate was reduced by 50% (Ekelund et al. 1993).

Biodegradation of NP, NP1EO, and NP2EO in soil-sludge mixtures and sludge slurries were examined by Madsen et al. (1998). Three different sludges collected from Danish WWTPs, either activated or anaerobically digested, were tested. After 28 days, no change in concentration was observed for NP, NP1EO or NP2EO; however, the analysis did not examine longer-chained nonylphenol ethoxylates, nor nonylphenoxy carboxylates. As such, it is possible that some degradation of NP and NP1-2EO did occur, but was masked by the transformation of longer-chained NPEs to short-chain NPEs and NP (Madsen et al. 1998). Two of the sludges were also examined for biodegradation by preparing slurries of the sludges and incubating them in the dark in closed beakers connected to an oxygen supply, and equipped with stirrers, at 15°C for 14 days. The pre-conditioned sludge bacteria in the slurry, possibly aided by desorption of sludge-bound contaminants through stirring, resulted in decreases of 70-80% of the initial levels of NP, NP1EO

and NP2EO for both sludges. How much of the decrease in NP and NPEs was due to mineralization is unknown since there also may have been formation of other degradation products such as NPECs.

Ultimate biodegradation of NP9EO has been observed in soil under aerobic conditions. During a nine week experimental period, 57% of the initially applied NP9EO was mineralized, including breakdown of the benzene ring, as confirmed through HPLC with UV detection (Hughes et al. 1996). Mineralization of nonylphenol has been observed in a variety of soil types, including agricultural soils of various textures, non-cultivated temperate soils, and soils from the Canadian tundra (Topp and Starratt 2000). The fact that biodegradation occurred in the arctic soils indicated that the microorganisms already possessed the biochemistry for nonylphenol metabolism without prior exposure and adaptation. The authors suggested that exposure of microorganisms to phenolic substances released by the decomposition of plant matter may have enabled them to degrade nonylphenol (Topp and Starratt 2000).

Mineralization of nonylphenol by soil bacteria can be inhibited by either an overabundance or a lack of water content (Topp and Starratt 2000). Temperature has also been observed to affect rates of nonylphenol mineralization in soil. Although biodegradation will occur over the temperature range of 4 to 30°C, at lower temperatures the rate decreases, and there is a greater lag period (Topp and Starratt 2000). Overall, the authors conclude that nonylphenol in land-applied sewage sludge would readily undergo biodegradation under the soil conditions of a normal Canadian growing season.

Wild and Jones (1992) suggest a half-life of <10 days for nonylphenol in sewage sludge. Data from Ekelund et al. (1993) indicate a half-life for NP in seawater of 56 days, while Sundaram and Szeto (1981) measured a freshwater half-life of about 16 days. Marcomini et al. (1988), studying degradation of NP in soil, measured a half-life of 8 days during the first 10 days after application, followed by a 90 day period in which the degradation rate slowed to give a half-life of 90 days. An analysis of data from various sources indicated that half-lives measured from the end of the lag phase to the beginning of the plateau for the NP degradation curves results in half-lives ranging from 6 to 26 days for water, soil, and water-sediment slurries (Naylor 2000).

Uptake/Elimination Pathways, and Metabolism

The degree to which a substance bioaccumulates in an organism is dependent on the relative rates of uptake and elimination. These processes can also have a bearing on the type, and magnitude, of toxic effect that a substance will have. Metabolism, or biotransformation, of a substance within an organism will produce metabolites that may be more or less toxic than the parent substance. Therefore, an understanding of the processes affecting a substance within an exposed organism will permit a greater understanding of the toxicological risks of that substance to the organism. A discussion of the physiological and metabolical pathways for NP and NPEs in fish, invertebrates, plants, and wildlife is provided below.

Numerous researchers have examined uptake and elimination pathways for nonylphenolic compounds in fish. Uptake occurs primarily through the gills with transport to the various tissues/organs via the blood (Granmo and Kollberg 1976; Arukwe et al. 2000b). For example, Granmo and Kollberg (1976) found that after only five minutes of exposure to NP10EO, high levels of the contaminant were observed in the gill tissue and blood of cod (*Gadus morhua*). Smaller amounts of NP10EO were also taken up through intestinal resorption. Uptake of nonylphenolic compounds is fairly rapid, with tissue concentrations reaching a steady state during the first 3 to 10 days of exposure (Granmo and Kollberg 1976; Ward and Boeri 1991c). Within a fish, nonylphenol can concentrate in the viscera at levels 1.6 to 7.1 times higher than in the carcass (Ward and Boeri 1991c). Once a stable equilibrium has been reached, the highest concentrations of nonylphenolics in fish can be found in the gall bladder, bile, pyloric caecae, intestine, and faeces (Granmo and Kollberg 1976; Lewis and Lech 1996; Coldham et al. 1998; Arukwe et al. 2000b). Intermediate levels of nonylphenolic compounds are found in the liver, kidney and fat tissue, and comparatively low concentrations can be found in the gills, heart,

gonad, muscle, spleen, skin, blood, eye and brain (Granmo and Kollberg 1976; Lewis and Lech 1996; Coldham et al. 1998; Arukwe et al. 2000b).

When fish exposed to nonylphenolic compounds are transferred to clean water, elimination of the contaminants will occur for all tissues, although there appears to be a longer time lag for the gall bladder, where concentrations of nonylphenolic substances have been observed to remain stable for extended periods (Granmo and Kollberg 1976; Coldham et al. 1998). For most of the tissues, Coldham et al. (1998) found that the elimination process was biphasic and fit a two compartment model. The highest rate of elimination occurs in the blood and gills (Granmo and Kollberg 1976), and the lowest rates of elimination occur in the liver, muscle, and skin tissues, as evidenced by the half-lives (Coldham et al. 1998). In rainbow trout (*Oncorhynchus mykiss*), the estimated half-life of NP in fat was 20 hours, in muscle it was 19 hours, and in the liver the half-life was six hours (Lewis and Lech 1996). Arukwe et al. (2000b) found that the half-lives for nonylphenol residues in the carcass, viscera and muscle of Atlantic salmon (*Salmo salar*) were all between 24 and 48 hours. For all tissues, rates of elimination are slower than rates of uptake (Coldham et al. 1998). The whole-body excretion half-life for juvenile Atlantic salmon exposed to technical grade *p*-nonylphenol was estimated at four days (McLeese et al. 1981). Some excretion is thought to occur via the kidney, with high levels of nonylphenolics being observed in the urine (Granmo and Kollberg 1976). Thibaut et al. (1999) gave rainbow trout a single dose of radioactively labelled nonylphenol and observed urinary elimination of 1.1% of the radioactivity after 24 hours, and 3.0% elimination through urine after 48 hours. In contrast, Coldham et al. (1998) suggest that the main pathway for elimination of nonylphenol follows a biliary/faecal route of excretion. Evidence for this pathway comes from the high equilibrium concentrations of nonylphenolics that have been observed in tissues associated with the biliary/faecal system (Granmo and Kollberg 1976; Lewis and Lech 1996; Coldham et al. 1998; Arukwe et al. 2000b). Ekelund et al. (1990) exposed three-spined sticklebacks (*Gasterosteus aculeatus*) to ^{14}C-labelled NP in a flow-through system with seawater for 16 days, followed by an elimination period of 32 days. They found that the sticklebacks reached steady states of bioaccumulation after about ten days and elimination of NP was quite rapid and almost complete after a few days.

Within the fish tissue, nonylphenol may be biotransformed into various metabolic products. Researchers have suggested that metabolic degradation of nonylphenol in fish occurs through oxidation and glucuronic acid formation in bile (Lewis and Lech 1996; Meldahl et al. 1996; Arukwe et al. 2000b). Through *in vitro* tests with rainbow trout hepatic microsomes, Meldahl et al. (1996) also observed metabolism of nonylphenol by hepatic cytochrome P450. Some possible metabolites of nonylphenol have been tentatively identified in rainbow trout as 9-(4-hydroxylphenyl)-nonanoic acid, 4-hydroxybenzoic acid, 3-(4-hydroxyphenyl) propionic acid, and 3-(4-hydroxylphenyl)-2-propenoic acid (Thibaut et al. 1999). The process of ω-oxidation followed by ß-oxidation has been proposed as the most likely biotransformation pathway (Thibaut et al. 1999).

There are few studies that have investigated uptake and elimination rates in invertebrates. Ekelund et al. (1990) exposed common blue mussels (*Mytilus edulis*) and common shrimp (*Crangon crangon*) to ^{14}C-labelled NP in a flow-through system with seawater for 16 days, followed by an elimination period of 32 days. The shrimp reached steady states of bioaccumulation after approximately ten days, however, with the mussels, steady state was not reached by the end of the 16 day exposure period. Elimination of NP from the shrimp took about four days, but was much slower in the mussels which still had significant concentrations present after 30 days in clean water. McLeese et al. (1980b) also found that common blue mussels have low uptake rates and have high excretion rates of nonylphenol.

There has been considerably greater investigation into the uptake/elimination and metabolism of nonylphenol in plants than in invertebrates. Much of the literature has focussed on agricultural plant species exposed to nonylphenolic compounds through contaminated soil. Based on the physical and chemical properties of nonylphenol, it has been hypothesized that high retention of nonylphenol at the root surface of plants would be likely, with only moderate uptake and

translocation occurring within the plants (Wild and Jones 1992). Kirchmann and Tengsved (1991) examined the grains of wheat plants grown in soil contaminated with nonylphenol due to sewage sludge application. They found no significant increase in nonylphenol concentrations in the grains, though were uncertain whether this indicated a lack of root uptake and transport to the grains, or rapid decomposition of the nonylphenol in the soil. Harms (1992) also did not observe any evidence of nonylphenol in the shoots of tomato plants whose roots were exposed to nonylphenol. In contrast, translocation of nonylphenol or its metabolites from roots to shoots within wheat, tomato and garden orache plants has been observed by other researchers (Bokern et al. 1998).

Bokern et al. (1996) examined the metabolism of radioactively labelled 4-*n*-nonylphenol in wheat cell suspension cultures. Metabolic products observed were 4-(hydroxy)- and 4-(dihydroxy)nonylphenols that were glucosylated at the phenolic OH-group and further glucosylated, glucuronidated, and acylated with acetic acid or malonic acid. Metabolism of nonylphenol was also examined in cell suspension cultures of *Daucus carota* (carrot) and *Atriplex hortensis* (garden orache) (Bokern and Harms 1997). Radiolabelled nonylphenol was taken up quickly in both cultures with 93-97% of the radioactivity disappearing from the culture medium after six hours, and concentrations reaching a steady state after 48 hours. Metabolism of nonylphenol within the cells occurred quickly. In the carrot cell cultures, 80% of the parent compound had been metabolized within 24 hours, and in the *A. hortensis* cells about 50% of the nonylphenol was metabolized after 30 hours (Bokern and Harms 1997). The authors also looked at the uptake and metabolism of nonylphenol in 14 different cell cultures (predominantly agricultural crop species). All of the species took up NP and metabolized it. Metabolites were ß-glucosides of differently hydroxylated nonylphenol, some of which were further acylated with, for example, glucuronic acid or malonic acid. Cell cultures of plants from similar taxonomic groups tended to produce similar metabolites (Bokern and Harms 1997). Distribution of radioactivity among the various cell wall fractions appeared to be species specific. In the majority of species, high concentrations were associated with the lignin, though in some species it was associated with the protein or hemicellulose. Although most plant species seem to readily take up nonylphenol (at least when exposed *in vitro*) and metabolize it, no mineralization of nonylphenol to CO_2 appears to occur in plants (Bokern et al. 1998).

Considerably less information exists on the uptake of nonylphenolic compounds by aquatic plants. In a littoral mesocosm study, nonylphenol applied to the water column was found to partition to macrophytes, with maximum concentrations of 4-NP observed in macrophytes within 1 to 2 days of application. The authors estimated the mean DT_{50} and DT_{95} for nonylphenol persistence in/on macrophytes at ten and 189 days, respectively, indicating persistence of nonylphenol in macrophytes (Heinis et al. 1999).

There is a paucity of data on uptake of nonylphenolic compounds by terrestrial animals. Based on the physical and chemical properties of nonylphenol, Wild and Jones (1992) have speculated that there is a moderate chance of transfer of nonylphenol to animal tissues through foliage ingestion, with less chance of transfer occurring through soil ingestion.

Bioaccumulation

Bioaccumulation is defined as the net accumulation of a substance by an organism as a result of uptake from all routes of exposure (e.g., water intake, food ingestion, dermal absorption, etc.). Bioconcentration also refers to net accumulation of a substance, but with respect to aquatic organisms, and involving only direct uptake from an aqueous solution. Thus, bioaccumulation includes the process of bioconcentration, but not vice versa. Nonylphenolic compounds have been evaluated for their potential to bioaccumulate or bioconcentrate both through direct investigations involving exposure of organisms to these substances under natural or artificial conditions, and through studies of their physical/chemical properties.

Numerous studies have examined bioaccumulation potential through examination of nonylphenol concentrations in aquatic biota relative to water concentrations, and nonylphenolic compounds

appear to bioaccumulate and bioconcentrate to a low or moderate degree. Several researchers have observed bioaccumulation of NP and NPEs in common blue mussels when placed in cages near industrial wastewater outflows or when exposed to NP (or wastewater containing NP and NPEs) under laboratory conditions (McLeese et al. 1980b; Wahlberg et al. 1990; Granmo et al. 1991a,b). Granmo et al. (1991b) found that the amount of bioaccumulation of NP, NP1EO, NP2EO, and NP3EO depended on the length of the ethoxylate chain with greater bioaccumulation of compounds with shorter ethoxylate chains (and therefore greater hydrophobicity). Ethoxylate chain length appears to affect bioconcentration in aquatic plants and algae as well. In their field study of the Glatt River, Switzerland, Ahel et al. (1993) found that although nonylphenol was present in the river water at a lower concentration (3.9 $\mu g \cdot L^{-1}$) than NP1EO or NP2EO (24 and 9.4 $\mu g \cdot L^{-1}$, respectively), it occurred at higher concentrations (2.5 to 38.0 $mg \cdot kg^{-1}$ dry weight) in the resident algae and macrophytes than the other two compounds (0.6 to 4.7 $mg \cdot kg^{-1}$ dry weight).

For the purposes of comparing bioaccumulation potentials from different studies, or across different substances and different species, bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) are calculated. Generally, BAFs are calculated in field studies and BCFs are determined in laboratory studies. Because BCFs do not include dietary uptake, they tend to underestimate the bioaccumulation potential of a substance. Table 3 lists BAF and BCF values that have been determined for NP and NPEs on a variety of aquatic species. In general, BAF and BCF values are higher for NP than for NPEs (i.e., BAFs and BCFs decrease with increasing ethoxylation). Considerable variation in BAF and BCF values are observed within taxa, and even within the same species, which makes it difficult to discern any significant differences in bioaccumulation between algae, plants, invertebrates, fish, and waterfowl. Under the Toxic Substances Management Policy (Government of Canada 1995) and under Canada's Persistence and Bioaccumulation Regulations (Government of Canada 2000b), a substance is considered bioaccumulative if its BAF (or BCF) is equal to or greater than 5000. Ahel et al. (1993) reported BAFs greater than 5000 in the algae *Cladophora glomerata*. Ekelund et al. (1990) calculated BCFs greater than 5000 in a crustacean, a mollusc, and a fish when based on lipid concentrations, but not when based on total wet weight. All other reported BAFs and BCFs fall below the critical value of 5000 (see Table 3), suggesting that nonylphenolic substances are not highly bioaccumulative.

Based on their physical and chemical properties, little bioaccumulation and bioconcentration of nonylphenolic compounds are likely to occur. The potential for a contaminant to bioaccumulate is largely affected by its hydrophobicity, and therefore is often evaluated based on its octanol-water partition coefficient (K_{ow}). Estimates of the log K_{ow} for nonylphenol of 4.7 (Barber et al. 1988), 4.48 (Ahel and Giger 1993b), 4.2 (McLeese et al. 1981), and 4.1 have been made (Wild and Jones 1992). Partition coefficients between *n*-octanol and water have also been determined for several of the smaller NPEs. The log K_{ow}s for NP1EO, NP2EO and NP3EO are estimated at 4.17, 4.21, and 4.20, respectively (Ahel and Giger 1993b). According to the Toxic Substances Management Policy (Government of Canada 1995) and Canada's Persistence and Bioaccumulation Regulations (Government of Canada 2000b), in the absence of BAF or BCF data, a substance may be considered as bioaccumulative if it has a log K_{ow} value equal to or greater than 5. As estimates of the log K_{ow} for nonylphenolic substances all fall below 5, this reinforces the conclusion based on BAF values that these substances are not bioaccumulative. Partition coefficients (K_{hw}) have also been determined for the partitioning of nonylphenolics between *n*-hexane and water. Log K_{hw}s for NP, NP1EO, and NP2EO were estimated at 3.7, 3.4, and 3.3, respectively (Ahel and Giger 1993b). The compounds generally had a lower solubility in hexane than in octanol. With both organic solvents, the difference of a single ethoxylate group had a negligible influence, with NP1EO and NP2EO having almost equal lipophilicity (Ahel and Giger 1993b). Although neither log K_{ow} nor log K_{hw} values have not been calculated for the higher NPEs, they are known to be less hydrophobic than nonylphenol and the lower molecular weight NPEs and would therefore have lower potential for bioaccumulation as well.

Substances that bioaccumulate or bioconcentrate may also have the potential to biomagnify. There is no evidence in the current literature to suggest that NP or NPEs biomagnify. For

example, Ahel et al. (1993) examined concentrations of NP, NP1EO and NP2EO in biota, at several trophic levels, of the Glatt River, Switzerland. For each of these substances, bioaccumulation factors in the algae *Cladophora glomerata* were significantly higher than the BAFs in three fish species (*Squalius cephalus*, *Barbus barbus*, and *Oncorhynchus mykiss*). Concentrations of nonylphenolic compounds detected in the tissues of a mallard duck (*Anas boscas*) were not significantly different from concentrations observed in fish tissues. Because the concentrations of NP and NPEs did not increase with increasing trophic level of organisms, the authors concluded that the compounds did not biomagnify.

Table 3. Bioconcentration and bioaccumulation factors for nonylphenol and nonylphenol ethoxylates in aquatic organisms.

Chemical	Species	Water type	BCF	BAF	Tissue	Reference
Plants/Algae						
NP	*Cladophora glomerata* (algae)	fresh	NR	10000 * 6600-7700	dw	Ahel et al. 1993
	Fontinalis antipyretica (macrophyte)	fresh	NR	1100	dw	Ahel et al. 1993
	Potamogeton crispus (macrophyte)	fresh	NR	600	dw	Ahel et al. 1993
NP1EO	*Cladophora glomerata* (algae)	fresh	NR	200 * 3500-5000	dw	Ahel et al. 1993
	Fontinalis antipyretica (macrophyte)	fresh	NR	40	dw	Ahel et al. 1993
	Potamogeton crispus (macrophyte)	fresh	NR	50	dw	Ahel et al. 1993
NP2EO	*Cladophora glomerata* (algae)	fresh	NR	500 * 1000-1800	dw	Ahel et al. 1993
	Fontinalis antipyretica (macrophyte)	fresh	NR	60	dw	Ahel et al. 1993
	Potamogeton crispus (macrophyte)	fresh	NR	200	dw	Ahel et al. 1993
Invertebrates						
NP	*Crangon crangon* (common shrimp)	marine	90,110	NR	based on total ww	Ekelund et al. 1990
			7500,5500	NR	lipid-based	Ekelund et al. 1990
	Mytilus edulis (common blue mussel)	marine	1.4-7.9 (K_1/K_2 = 10)	NR	NR	McLeese et al. 1980b
			NR	340	NR	Granmo et al. 1991b
			2740,4120	NR	based on total ww	Ekelund et al. 1990
			169300, 216600	NR	lipid-based	Ekelund et al. 1990
NP1EO	*Mytilus edulis* (common blue mussel)	marine	NR	170	NR	Granmo et al. 1991b
NP2EO	*Mytilus edulis* (common blue mussel)	marine	NR	100	NR	Granmo et al. 1991b
NP3EO	*Mytilus edulis* (common blue mussel)	marine	NR	60	NR	Granmo et al. 1991b

Table 3 (continued). Bioconcentration and bioaccumulation factors for nonylphenol and nonylphenol ethoxylates in aquatic organisms.

Chemical	Species	Water type	BCF	BAF	Tissue	Reference
Fish						
NP	*Barbus barbus* (barb)	fresh	NR	92	muscle, gut, liver, heart, roe (dw)	Ahel et al. 1993
	Cyprinus carpio (carp)	fresh	2.5-3.3 0.9-2.2	NR	NR	CITI 1992 as cited in Staples et al. 1998
	Gasterosteus aculeatus (3-spined stickleback)	marine	1200,1300	NR	based on total ww	Ekelund et al. 1990
			17800, 16700	NR	lipid-based	Ekelund et al. 1990
	Lepomis macrochirus (bluegill sunfish)	fresh	220	NR	ww	Brooke 1993b
			NR	87	ww	Liber et al. 1999a
	Oncorhynchus mykiss (rainbow trout)	fresh	21-29	NR	carcass	Lewis and Lech 1996
			90-110	NR	viscera minus gall bladder/ bile	Lewis and Lech 1996
			NR	224	muscle, gut (dw)	Ahel et al. 1993
	Pimephales promelas (fathead minnow)	fresh	271, 344	NR	whole fish	Ward and Boeri 1991c
			741	NR	ww	Brooke 1993b
	Salmo salar (Atlantic salmon)	marine	75	NR	NR	McLeese et al. 1981
			190	NR	NR	McLeese et al. 1981
			235	NR	NR	McLeese et al. 1981
			280	NR	NR	McLeese et al. 1981
	*Squalius cephalus*** (chub)	fresh	NR	246	muscle, gut, liver, gills (dw)	Ahel et al. 1993
NP1EO	*Barbus barbus* (barb)	fresh	NR	31	muscle, gut, liver, gills, roe, heart (dw)	Ahel et al. 1993
	Oncorhynchus mykiss (rainbow trout)	fresh	NR	155	muscle, gut (dw)	Ahel et al. 1993
	*Squalius cephalus*** (chub)	fresh	NR	263	muscle, gut, liver, gills (dw)	Ahel et al. 1993

Table 3 (continued). Bioconcentration and bioaccumulation factors for nonylphenol and nonylphenol ethoxylates in aquatic organisms.

Chemical	Species	Water type	BCF	BAF	Tissue	Reference
NP2EO	*Barbus barbus* (barb)	fresh	NR	88	muscle, gut, liver (dw)	Ahel et al. 1993
	Oncorhynchus mykiss (rainbow trout)	fresh	NR	162	muscle, gut (dw)	Ahel et al. 1993
	*Squalius cephalus*** (chub)	fresh	NR	73	muscle, gut, liver, gills (dw)	Ahel et al. 1993
Wildlife						
NP	*Anas boscas* (mallard duck)	fresh	NR	308	muscle (dw)	Ahel et al. 1993
NP1EO	*Anas boscas* (mallard duck)	fresh	NR	88	muscle (dw)	Ahel et al. 1993
NP2EO	*Anas boscas* (mallard duck)	fresh	NR	37	muscle (dw)	Ahel et al. 1993

NR = not reported
ww = wet weight
dw = dry weight

* the authors (Ahel et al. 1993) note that due to various sources of uncertainty these calculated BAFs for *Cladophora glomerata* are only intended to provide an approximation of the order of magnitude and should not be interpreted as exact bioaccumulation factors
** the more commonly accepted name for this species is *Leuciscus cephalus*

Toxicity

Mode of Action

Although extensive research has been conducted on the toxic effects of nonylphenolic compounds, much less is known about their actual mode of toxicity. It has been suggested that the mode of toxicity for nonylphenol and the lower chain length NPEs and NPECs is narcosis, while toxicity from longer chain length NPEs may be due to physical surfactant effects (Fay et al. 2000; Servos et al. 2000). It is also likely that the mode of toxicity will depend on the dose, with high concentrations above the critical micelle concentration resulting in more effects due to physical surfactancy. Nonylphenolic substances may also act as estrogen agonists to disrupt endocrine functions.

Narcosis

Narcosis is a nonspecific, reversible mode of toxic action in which the presence of hydrophobic organic chemicals causes a disruption of cellular activity, possibly through binding to proteins in the cell membrane, or through "swelling" of the lipid portion of cell membranes (Rand 1995). Numerous researchers have observed changes in the cellular membranes of organisms exposed to NP and NPEs, thus giving support to the theory that these substances exert a narcotic-type effect. For example, increased cell membrane permeability occurs in plants exposed to nonylphenol (Silcox and Holloway 1986). Linsel et al. (1990) found that NPEs caused membrane depolarization affecting electron transport across the plasmalemma of the aquatic plant *Egeria densa*. Octylphenol ethoxylates, that likely have a similar mode of toxicity to their nonylphenol counterparts, are thought to act on aquatic macrophytes at the cellular membrane (Caux and Weinberger 1993). It has been proposed that at low surfactant concentrations (i.e., below the critical micelle concentration) the surfactant molecules mimic membrane phospholipids and intercalate in the phospholipid bilayer of the cell membrane causing compression of the lipid constituents, thereby inducing fatty acid desaturate enzymes to render membranes more fluid (Caux and Weinberger 1993). In unicellular algae, nonylphenol concentrations greater than 1000 $\mu g \cdot L^{-1}$ cause membrane disorganization including crennulation, vesiculation and pinocytotic invaginations of the nuclear membrane, as well as cytoplasm friability and distortion of flagellae indicative of microtubular damage (Weinberger and Rea 1981; Weinberger and Greenhalgh 1984). Tree seedlings grown in aqueous solutions of nonylphenol display excessive swelling of cell mitochondria, loss of chloroplast integrity, and convolution of the plasmalemma at concentrations of 1000 $\mu g \cdot L^{-1}$ (Weinberger and Greenhalgh 1984). Massive K^+ ion leakage from the roots of tree seedlings also occurs at NP concentrations of 1500 $\mu g \cdot L^{-1}$ (Weinberger and Greenhalgh 1984).

There are indications that nonylphenolic compounds may alter the permeability of cellular membranes in animals. For example, tissues of saltwater mussels became swollen when exposed to a nonylphenol polyethoxylate. The authors concluded that the swelling was likely caused by some nonspecific damage to the cell membranes that could have affected the osmoregulatory capacity of the tissues (Braaten et al. 1972). At a very high concentration (63,000 $\mu g \cdot L^{-1}$) NP10EO also caused an increase in ion permeability in the gill epithelial cells of a rainbow trout perfused head (Pärt et al. 1985). A decreased oxygen diffusion capacity was measured in the gills, possibly because the altered permeability caused swelling of the epithelium (Pärt et al. 1985). There was also an increase in the vascular resistance of the gills that may have been caused by an inhibition of the adrenergic dilatation of the gill vasculature by the surfactant (Pärt et al. 1985).

Nonylphenol also causes vasodilation in the vascular smooth muscle of rats by inhibiting Ca^{2+} influx through L-type Ca^{2+} channels in the smooth muscle cells (Ruehlmann et al. 1998). The sarcoplasmic reticulum (Ca^{2+} - Mg^{2+})-ATPase may be another specific target site of nonylphenol action in animal cells (Micheiangeli et al. 1990). At high concentrations (5 to15 µM), nonylphenol inhibits the ATPase activity, while at low concentrations (1 to 3 µM) it increases ATPase activity

(Michelangeli et al. 1990). Nonylphenol may, therefore, affect other P-type ATPase electroenzymes such as the plasma membrane H^+-ATPase of plants and fungi (Karley et al. 1997). It appears that NP may partition into the mitochondrial membranes of fungi and induce ionophoretic uncoupling of respiration (Karley et al. 1997). This process would restrict the cytosolic ATP supply, thus indirectly inhibiting the plasma membrane H^+-ATPase that is essential in maintaining cytosolic ionic homeostasis (Karley et al. 1997). Weinberger and Greenhalgh (1984) found that NP at 6.25 $\mu g \cdot L^{-1}$ causes disruption in the normal sequence of ATP production in birch and pine seedlings.

QSAR modelling also supports the theory that acute toxicity of APEs follows a narcotic-type mechanism that is likely to be intermediate with respect to the nonpolar and polar narcosis syndromes (Schüürmann 1991). Although information is not available for NP itself, QSAR modelling has shown that other APs, such as 4-propylphenol and *p-tert*-pentylphenol, also have a mode of action that is between nonpolar (type I) and polar (type II) narcosis (Veith and Broderius 1990).

Endocrine Disruption

There is considerable evidence to suggest that nonylphenol is an endocrine disrupting chemical (EDC) with estrogenic properties. The discovery was first made unintentionally when Soto et al. (1991), who were working with human MCF_7 breast tumour cells, found that the use of a new brand of polystyrene centrifuge tubes resulted in cell proliferation. They were able to extract the substance being released from the polystyrene using methanol, purify it through flash chromatography and reverse-phase HPLC, and identify it by GC-MS as nonylphenol. Further tests showed that the nonylphenol induced progesterone receptors, as well as cell proliferation in the MCF_7 cells, and it induced mitotic activity in rat endometrium - all indicators of estrogenic activity (Soto et al. 1991).

It has since been determined that NP, NP2EO, and NP1EC mimic the effects of 17ß-estradiol by binding to the estrogen receptor, thereby acting as an estrogen agonist (White et al. 1994). Moreover, these compounds will competitively displace 17ß-estradiol from binding to the estrogen receptor, suggesting that they have intrinsic estrogenic activity (White et al. 1994). Estrogen receptor competitive binding assays have been conducted with rainbow trout hepatocyte cultures to ascertain relative potencies of the various nonylphenolics. Relative potencies of nonylphenol compared to 17ß-estradiol have been measured at 1.0×10^{-3} (Tremblay et al. 1995) and 2.5×10^{-4} (Tremblay and Van Der Kraak 1998). Estrogenicity of NP and NP2EO is similar, while NP1EC shows slightly greater estrogenicity (White et al. 1994). White et al. (1994) found that APEs with more than 3 EO groups had little or no estrogenicity. This seems to indicate that the larger NPEs that are used commercially are unlikely to act as estrogen agonists in their parent form.

Numerous studies have since examined the estrogenicity of nonylphenol in fish through vitellogenin production. Vitellogenin is a lipoglycophosphoprotein that is normally produced in the liver of sexually mature female fish under estrogenic control (Jobling and Sumpter 1993; Bjerregaard et al. 1998). Vitellogenin secreted by the liver is transported via the blood to the ovaries where it forms the major constituent of the yolk in oocytes. Male fish possess the gene for vitellogenin, but normally do not express it due to low concentrations of estrogen in their systems. Treatment of male fish with estrogen, or chemicals with estrogen-like activity, will induce vitellogenin production and blood concentrations of vitellogenin will increase rapidly. Because this vitellogenin response is directly related to the binding of chemicals to the estrogen receptor, it may be used as a biomarker for estrogenicity (Bjerregaard et al. 1998).

Following intraperitoneal injections of nonylphenol (at concentrations ranging from 10 to 200 $mg \cdot kg^{-1}$ body weight), elevated vitellogenin levels (as much as several hundred-fold and as high as 8000 $mg \cdot L^{-1}$) have been detected in the blood plasma of various fish species, including rainbow trout (Christiansen et al. 1998a), flounder (*Platichthys flesus*; Bjerregaard et al. 1998), Atlantic salmon (Yadetie et al. 1999), and eelpouts (*Zoarces viviparus*; Christiansen et al. 1998b). Elevation of vitellogenin levels from control levels of 30 $mg \cdot L^{-1}$ to 40000 $mg \cdot L^{-1}$ of plasma have

also been observed in eelpouts exposed to nonylphenol (at 1000 $\mu g \cdot L^{-1}$) through the water column (Korsgaard and Pedersen 1998). The increase in vitellogenin level observed in the plasma shows a dose-dependent relationship with the amount of nonylphenol administered (Bjerregaard et al. 1998; Korsgaard and Pedersen 1998; Yadetie et al. 1999).

Arukwe et al. (1997b) have suggested that eggshell zona radiata proteins (*Zrp*) are even more sensitive than vitellogenin as biomarkers of estrogenic activity by nonylphenol in fish. Like vitellogenin, *Zrp* are produced in the liver in response to estrogen stimulation, and are transported to the ovary where they form eggshells, while vitellogenin forms the yolks. Juvenile Atlantic salmon injected intraperitoneally with nonylphenol have shown significantly elevated levels of *Zrp* as well as vitellogenin (Arukwe et al. 1998; Yadetie et al. 1999).

Estrogenicity of nonylphenolic compounds has also been investigated through *in vitro* assays in which vitellogenin induction in fish hepatocytes has been assessed. Jobling and Sumpter (1993) assessed the estrogenic capacities of NP, NP1EC, NP2EO, NP9EO, and NP40EO using an *in vitro* bioassay with cultures of male rainbow trout hepatocytes. Treatment with NP2EO and NP1EC resulted in a vitellogenin production 40-50 times higher than that of controls with mean ED_{50}s of 17.27 µM (5326 $\mu g \cdot L^{-1}$) and 15.25 µM (4916 $\mu g \cdot L^{-1}$), respectively. NP and NP9EO caused an 18-20 fold increase in vitellogenin production with mean ED_{50}s of 16.15 µM (3558 $\mu g \cdot L^{-1}$) and 82.31 µM (50800 $\mu g \cdot L^{-1}$), respectively. All of these substances became cytotoxic at concentrations between 50 and 200 µM, with NP being more toxic than the ethoxylates. The concentration of vitellogenin found in the NP40EO treatment was not significantly different from the control. The authors noted that the potency of the polyethoxylate compounds seems to decrease with increasing length of the ethoxylate chain. When NP was added to rainbow trout hepatocytes with Tamoxifen (which inhibits binding to the estrogen receptor site), the vitellogenin production was significantly lower than when NP was added alone. This result further confirms that NP also acts via the estrogen receptor in a manner similar to estradiol itself (Jobling and Sumpter 1993).

Rainbow trout hepatocytes were treated with estradiol in both the presence and absence of nonylphenol to assess the effects on induction of vitellogenin mRNA and estrogen receptor mRNA (Flouriot et al. 1995). No synergistic, additive, or repressive effects were observed. Knudsen et al. (1998) also found no evidence of synergism in the induction of zona radiata proteins or estrogen receptor modulation when octylphenol and estradiol were administered simultaneously to rainbow trout through intraperitoneal injections. When five estrogenic chemicals (nonylphenol, octylphenol, *o,p'*-DDT, Aroclor 1221, and bisphenol A) were administered together to rainbow trout hepatocytes, a greater induction of vitellogenin was observed when compared with the effects of each chemical when administered singly (Sumpter and Jobling 1995).

Aside from vitellogenesis, other estrogenic effects have also been observed in fish. Intraperitoneal injection of male eelpouts with 10 and 100 $\mu g \cdot g^{-1}$ body weight per week of nonylphenol resulted in reduced gonadosomatic indices and severe histological effects on the testicular structure (Christiansen et al. 1998b).

In a study comparing historical pesticide application data with recreational catch data from Atlantic Canada, epidemiological evidence showed a relationship between use of Matacil 1.8D (an insecticide formulated with NP) and a decline in catches of Atlantic salmon, *Salmo salar* (Fairchild et al. 1999). Similar declines were not observed with the application of other Matacil that did not contain NP, indicating that NP was the causal agent for the reduced fish abundance. Because the timing of the pesticide spraying coincided with the final stages of salmon smoltification, the authors speculated that endocrine disruption of smoltification may have led the lower catches (Fairchild et al. 1999). Laboratory studies with juvenile Atlantic salmon have also indicated that NP may affect the smolting and osmoregulatory processes of anadromous fish when they are exposed to seawater (Madsen et al. 1997; Brown et al. 1998).

Endocrine disruption and estrogenic effects of NP, NP2EO, NP1EC and NP2EC have also been observed in a variety of organisms other than fish. Exposure to nonylphenol has been found to increase the production of cypris major protein in larvae of the marine barnacle *Balanus amphitrite* (Billinghurst et al. 2000). Cypris major protein is required for successful larval settlement and juvenile development.

Immature female rats showed dose-dependent increases in uterine mass, increased protein and DNA contents of the uterus, and increased levels of the uterine peroxidase enzyme when injected intraperitoneally with nonylphenol at concentrations greater than 1 mg per animal (Lee and Lee 1996). This estrogenic response due to nonylphenol exposure was approximately $1x10^{-3}$ to $2x10^{-3}$ times as potent as that elicited by estradiol. Administration of an estrogen antagonist blocked the effects of NP, confirming that the action of nonylphenol in rats is through the estrogen receptor (Lee and Lee 1996). The authors also found that there is direct competition between NP and estradiol. Because NP is less efficient than estradiol, its presence reduces the estrogenic effects of estradiol. Chapin et al. (1999) examined effects of NP administered through the diet on several generations of rats. Effects indicative of an estrogenic response included vaginal opening at a younger age when exposed to NP at 650 $mg \cdot kg^{-1}$ food, and increased estrous cycle length at an NP concentration of 2000 $mg \cdot kg^{-1}$ food.

Using a recombinant yeast strain, Routledge and Sumpter (1996) examined the ability of nonylphenolic compounds to stimulate transcriptional activity of the human estrogen receptor (hER). NP, NP1EC, NP2EC and NP2EO were all found to produce dose-dependent elevations of estrogenic activity. This yeast estrogen screening assay was repeated by Metcalfe et al. (2001), confirming the estrogenic responses to NP, NP1EO and NP2EO. With NP1EC and NP2EC, however, Metcalfe et al. (2001) observed no estrogenic response. Similar results were obtained in assays with Japanese medaka (*Oryzias latipes*) in which a mixture of NP1EO and NP2EO was observed to alter gonadal development, while no evidence of estrogenicity was observed for a mixture of NP1EC and NP2EC (Metcalfe et al. 2001). The contradiction between these two studies indicates that further work is needed to confirm whether or not NPECs produce estrogenic responses.

White et al. (1994) found that NP, NP2EO and NP1EC were estrogenic in mammalian (mouse, human) and avian (chicken) cells. Lutz and Kloas (1999) compared the estrogenic binding affinities for several compounds by examining the competitive displacement of estradiol in cytosolic fractions from the livers of the amphibian *Xenopus laevis*. They found that NP had a higher binding affinity than octylphenol. Fifty percent displacement of 18.9 nM estradiol occurred at 7424 $\mu g \cdot L^{-1}$ NP and at 16153 $\mu g \cdot L^{-1}$ OP.

Besides acting as an estrogen agonist, it appears that nonylphenol may also be capable of interacting with androgen receptors, though with a lower affinity than for estrogen receptors (Gray and Kelce 1996). Both NP and NPEs have been found to reduce the elimination of glucose- and sulphate-conjugated testosterone metabolites while increasing the elimination of oxido-reduced derivatives of testosterone and increasing metabolic androgenization in daphnids (Baldwin et al. 1997, 1998).

A brief discussion of the ecological relevance of endocrine disruption, and whether such endpoints should be used in the development of environmental quality guidelines, is presented in Annex I.

Physical Surfactancy

Apart from interactions with cellular membranes and membrane transport proteins, or endocrinal effects (as discussed in the next section), the only other mode of toxicity for nonylphenol mentioned in the literature deals with its physical surfactancy. For insects with an aquatic larval stage, such as mayflies and caddisflies, decreased adult emergence has been observed during and immediately after NP application to water. The decreased emergence has been attributed to the surfactant properties of NP that may have rendered the insects "wettable", thus preventing them from escaping the surface tension (Schmude et al. 1999). Effects due to physical

surfactancy are most likely to occur if concentrations of NP or NPEs are sufficiently high for micelles to form. Typical environmental concentrations in Canada are generally below critical micelle concentrations for these substances, and therefore this mode of toxicity is not likely to occur often (see the list of critical micelle concentrations in Chapter 1 and the discussion of Canadian environmental concentrations in Chapter 2).

Toxic Effects

Toxicity of nonylphenol and nonylphenol ethoxylates has been observed in diverse biological taxa including bacteria, fungi, protozoans, algae, aquatic macrophytes, terrestrial plants, crustaceans, insects, molluscs, oligochaetes, annelids, rotifers, nematodes, collembolans, fish, amphibians, and mammals. Researchers have also determined the toxicity of NP and NPEs in many of these organisms at various developmental stages. Observed toxic effects include mortality, growth inhibition, reduced fecundity, reduced germination, reduced abundance, reduced egg viability, reduced larval settlement, reduced emergence, reduced hatch rate, delayed maturity, reduced photosynthesis, immobilization, lethargy, loss of equilibrium, membrane disruption, induced vitellogenesis, zona radiata induction, modified sex ratios, induction of testis-ova, and changes in soil nutrient cycling (e.g., nitrification, nitrogen-fixation by bacteria).

Comprehensive lists of all fresh water, marine water, sediment, and soil toxicity data found in the literature have been compiled in Appendices I, II, III, and IV, respectively. Within each of these appendices, the data have been organized under broad taxonomic headings (e.g., microorganisms, plants, invertebrates, fish, amphibians, etc.). The data have also been grouped by compound (i.e., NP, NPEs, NPECs). Toxicity data relevant to the derivation of the water, sediment, and soil quality guidelines are discussed within Chapters 5, 6 and 7, respectively. Below is a brief summary of the major, over-arching trends in the toxicity data.

Comparing LC_{50} and EC_{50} values across the various taxonomic groupings, there appears to be a great deal of overlap. Toxic NP concentrations are quite comparable for most invertebrates and fish, while plants and algae, in general, are slightly less sensitive. Comparing across media, NP is approximately one hundred times more toxic in water (either freshwater or marine) than it is in sediment or soil. The bulk of toxic responses in water fall within the range of 0.1 to 10 ppm (i.e., 100 to 10000 $\mu g \cdot L^{-1}$; see Appendix I and II) while most toxic responses in sediment and soil are within the range of 10 to 1000 ppm (i.e., 10 to 1000 $mg \cdot kg^{-1}$ dry weight; see Appendix III and IV). This difference likely reflects the lower bioavailability of the compounds in soil and sediment due to particle adsorption. There are large differences in toxicity among the various nonylphenolic ethoxamers. For example, LC_{50}s for freshwater exposure of Japanese killifish, *Oryzias latipes*, to NP, NP1EO, NP6.4EO, NP9EO, and NP16.6EO are 1400, 3000, 5400, 12000, and 110000 $\mu g \cdot L^{-1}$, respectively (Yoshimura 1986). In general, toxicity decreases with increasing ethoxylation.

It appears that the concentrations of NP required to elicit estrogenic effects are comparable to concentrations at which other chronic toxic effects are observed. Estimates of NP chronic toxicity in freshwater fish, for conventional endpoints, range from 6.0 $\mu g \cdot L^{-1}$ (*Oncorhynchus mykiss* 91-day NOEC growth; Brooke 1993a) to 460 $\mu g \cdot L^{-1}$ (*Oryzias latipes* 17-day LC_{50} embryotoxicity; Gray and Metcalfe 1997). For estrogenicity endpoints, the chronic toxicity estimates are of a similar magnitude, ranging from 10 $\mu g \cdot L^{-1}$ (*Oryzias latipes* 3-month NOEC for testis-ova induction; Gray and Metcalfe 1997; also *Oncorhynchus mykiss* 72-hour LOEC vitellogenin induction; Lech et al. 1996) to 100 $\mu g \cdot L^{-1}$ (*Oryzias latipes* 3-month LOEC for sex ratio change; Gray and Metcalfe 1997). These data indicate that guidelines for nonylphenolic substances set at concentrations that protect against conventional toxicity endpoints are likely to protect against most endocrine disrupting effects as well.

Mixtures and the TEQ Approach

Nonylphenol, nonylphenol ethoxylates, and nonylphenol ethoxycarboxylates typically occur together in the aquatic environment as mixtures. Therefore, for the purpose of assessing acceptable levels of these substances in water, sediment or soil, it is more useful to develop guidelines that consider their combined effects. This can be accomplished through the use of a toxic equivalency (TEQ) approach.

The TEQ approach is used to characterize the toxicity of a mixture of compounds by expressing the toxicities of each individual compound in common terms, and summing them. This method is commonly applied to PCBs, dioxins, and furans, and has previously been used in the development of Canadian Environmental Quality Guidelines for these chemicals (Environment Canada 2000a,b). Application of the TEQ approach requires that all components of a mixture have a common mode of toxicity such that their effects are additive. Nonylphenolic substances meet this requirement. At typical environmental concentrations, and for conventional toxicity endpoints, the mode of toxicity for nonylphenol and for at least the lower ethoxylate chain length NPEs and NPECs is narcosis (see previous discussion). If these substances are all acting through narcosis, then additivity of their effects (as opposed to synergism or antagonism) is most likely (Servos et al. 2000).

To apply a TEQ approach, toxic equivalency factors (TEFs) are determined for each individual compound in a mixture by expressing the potency of each compound relative to the most toxic compound in the mixture. For PCBs, dioxins, and furans, TEFs are measured relative to the highly toxic 2,3,7,8-TCDD (van den Berg et al. 1998). Each compound's concentration in the mixture is then multiplied by its TEF to obtain its toxic equivalent (TEQ) for that sample, relative to 2,3,7,8-TCDD. The overall toxicity of the mixture is then calculated by summing the TEQs for all compounds contained in the mixture.

We propose that a similar TEQ approach be applied towards mixtures of nonylphenolic substances. We are not recommending the incorporation of nonylphenolic substances into the same TEQ system used for PCBs, dioxins, and furans; the mode of toxicity for these two groups of substances are different, so their toxicities cannot be compared (Roe et al. 2000; Environment Canada 2000a). Rather we are suggesting that a separate set of nonylphenol TEF values (NP TEFs) be used. Several researchers have studied the relative toxicity of various nonylphenol ethoxylates and found that toxicity increases with decreasing length of the ethoxylate chain (e.g., Maxwell and Piper 1968; Macek and Krzeminski 1975; Yoshimura 1986; Miossec and Bocquéné 1986; Hall et al. 1989). Therefore the degradation products of NPEs (e.g., NP, NP1EO, NP2EO) are more toxic than the parent compounds. In the application of a TEQ approach to these compounds then, potency should be expressed relative to nonylphenol, the most toxic substance.

Relative toxicity values, or NP TEFs, for various nonylphenolic compounds were calculated for the CEPA assessment of nonylphenol (Servos et al. 2000) and are presented in Table 4. These values were derived based on a broad dataset that included both acute and chronic studies on a range of vertebrate and invertebrate species. Reported toxic concentrations for the various nonylphenolic compounds were matched up against similar endpoints for nonylphenol with the same species, and, where possible, from the same laboratory; then a relative toxicity ratio was calculated. From the resulting list of relative toxicity values for each group of compounds, a mean relative toxicity value (TEF) was calculated, with more weight given to those studies deemed to be of higher quality.

These TEFs represent an initial attempt to define the relative toxicity of groups of nonylphenolic compounds, rather than for each individual ethoxamer. Future work to develop and validate more specific TEF values is needed, but, in the interim, it is recommended that these more general TEFs be applied. Also, TEF values for NPEs containing 3 to 8 ethoxylate groups were not estimated in the CEPA assessment because there have been very few toxicity tests conducted with these substances. Instead, we have provided our own estimate. As toxicity is known to generally decrease with increasing number of ethoxylate groups, the relative toxicity of NP3EO-

NP8EO likely lies somewhere between the TEFs of 0.5 (for NP2EO) and 0.005 (for NP*n*EO where n≥9). Therefore, for NPEs with 3 to 8 EO groups, until sufficient toxicity data is available to estimate relative potencies, it is recommended that a conservative TEF of 0.5 be adopted, with the caveat that the use of this TEF may overestimate the toxicity of a mixture.

The total concentration of NP and NP-equivalents in a sample can be calculated using the equation:

$$\text{Total concentration} = \sum (C_x \times TEF)$$

where,

C_x = concentration of each nonylphenolic compound
TEF = toxic equivalency factor for each particular nonylphenolic compound.

In the assessment of a mixture of nonylphenolic compounds, the environmental concentrations of NPEs and NPECs should be expressed in terms of NP equivalents, and the sum of these concentrations should then be compared against the guidelines developed in this document.

The NP TEF values determined in the CEPA assessment (Servos et al. 2000) were based on toxicity to aquatic species which reside in the water column, but they may also be applied towards the soil and sediment guidelines for NP and NPEs. Narcosis appears to be a common mode of toxicity for nonylphenolic substances across all taxonomic groups (see earlier discussion under "Modes of Toxicity"), so the NP TEF values should be applicable to plants, invertebrates, fish, mammals, etc., regardless of whether they are water-, sediment-, or soil-dwelling species.

If octylphenol, octylphenol ethoxylates, or octylphenol ethoxycarboxylates are present in environmental samples, it is recommended that these substances also be considered in the calculation of total NP-equivalents. OP and OPEs are very similar in structure and behaviour to NP and NPEs, and likely act through a similar toxic mode of action. Servos et al. (2000) have suggested TEF values relative to NP for OP, OP1EC and OP2EC at 1.0, 0.005, and 0.005, respectively. We would recommend that for OP*n*EOs, the same TEF values for the corresponding NP*n*EO analogs could be used.

The TEQ approach has not been previously applied towards NP and NPEs, so the guidelines currently available from other jurisdictions, as described in Chapter 4, are either based solely on a single ethoxamer (e.g. NP or NP9EO), or they are based on the straight sum of NP and NPE concentrations with no regard for relative toxicities among the ethoxamers. The Canadian Environmental Quality Guidelines for NP and NPEs, developed in Chapters 5, 6, and 7, are expressed on a TEQ basis.

Table 4. Summary of relative toxicity of nonylphenolic and related compounds.

Chemical	Toxic equivalency factors (TEFs) relative to NP
NP	1
NP1EO	0.5
NP2EO	0.5
NP*n*EO ($3 \leq n \leq 8$)	0.5*
NP*n*EO ($n \geq 9$)	0.005
NP1EC	0.005
NP2EC	0.005
OP	1
OP*n*EO ($1 \leq n \leq 8$)	0.5*
OP*n*EO ($n \geq 9$)	0.005*
OP1EC	0.005
OP2EC	0.005

Adapted from Servos et al. (2000) except where otherwise noted.
* conservative estimation by the authors of this document

CHAPTER 4. GUIDELINES FROM OTHER JURISDICTIONS

Water

Freshwater

Tables 5 and 6 provide summaries of existing freshwater guidelines, objectives, criteria, standards, and environmental concern levels for nonylphenol (NP) and nonylphenol ethoxylates (NPEs), respectively, from various jurisdictions both within and outside of Canada.

In Canada, the Quebec provincial government has established interim water quality criteria for both nonylphenol (CAS 25154-52-3) and nonylphenol ethoxylates (CAS 9016-45-9). For nonylphenol, the interim criteria (developed in 1996) for the protection of aquatic life from acute and chronic toxicity are 6.8 $\mu g \cdot L^{-1}$ and 0.3 $\mu g \cdot L^{-1}$, respectively. The interim criteria for nonylphenol ethoxylates (established in 1991) for the protection of aquatic life from acute and chronic toxicity are 470 $\mu g \cdot L^{-1}$ and 21 $\mu g \cdot L^{-1}$, respectively (MEF 1998). These criteria have recently undergone review, and new criteria are scheduled for release in 2000 (Guay 2000). For nonylphenol, new acute and chronic final criteria will be 29 $\mu g \cdot L^{-1}$ and 6.0 $\mu g \cdot L^{-1}$, respectively. For NP9EO, provisional acute and chronic criteria will be 280 $\mu g \cdot L^{-1}$ and 12 $\mu g \cdot L^{-1}$, respectively. For NP1EC, provisional acute and chronic criteria will be 100 $\mu g \cdot L^{-1}$ and 4 $\mu g \cdot L^{-1}$, respectively. It was decided that there is currently insufficient data available to derive criteria for NP1EO, NP1.5EO, NP2EO, NP10EO, and NP2EC.

The province of Ontario has an interim provincial water quality objective for nonylphenol of 0.04 $\mu g \cdot L^{-1}$. This Ontario objective was based on the 48h LC_{50} of 100 $\mu g \cdot L^{-1}$ for *Daphnia pulex* (EPS 1981 as cited in NRCC 1982), that was then divided by a highly conservative uncertainty factor of 2332 due to the scarcity of toxicological data at the time (OMOE 1991). Many more aquatic toxicity data are now available.

Outside of Canada, Denmark has an environmental quality standard for freshwater concentrations of NP and NPEs combined. Australia and New Zealand have an environmental concern level for nonylphenol in fresh water, and the U.S.A. has developed draft numbers for both acute and chronic ambient aquatic life water quality criteria for nonylphenol.

Marine/Estuarine

A summary of existing marine and estuarine water quality guidelines for NP from other jurisdictions are provided in Table 7. There are currently no jurisdictions in Canada with marine water quality guidelines for NP or NPEs. Australia and New Zealand have an environmental concern level for NP in marine waters, while the U.S.A. has both acute and chronic ambient aquatic life water quality criteria for NP in marine waters.

Sediment

There are no jurisdictions within Canada, nor outside of Canada, which have guidelines for NP or NPEs in either freshwater or marine sediment.

Soil

No guidelines for nonylphenol in soil currently exist for other jurisdictions within Canada. Outside of Canada, only Denmark has a soil quality criterion for nonylphenol in soil (Table 8). Denmark also has a guideline for the total concentration of NP, NP1EO, and NP2EO that may be present in sludge which is intended to be applied to agricultural soils. Sweden also has a guideline on the allowable concentration of NP in sludge used as an agricultural fertilizer (see Table 8).

Table 5. Freshwater quality guidelines for nonylphenol from other jurisdictions.

JURISDICTION	GUIDELINE ($\mu g \cdot L^{-1}$)	LEVEL OF PROTECTION	REFERENCE
Ontario, Canada	0.04	interim provincial water quality objective for the protection of aquatic life	OMEE 1994
Australia / New Zealand [1]	0.2	environmental concern level	See footnote
Quebec, Canada [2]	0.3	interim water quality criteria for the protection of aquatic life (chronic exposure)	MEF 1998
Denmark	1.0 (NP and NPEs)	environmental quality standard	Renner 1997
U.S.A. [3]	5.9 (4-d average)	ambient aquatic life water quality criteria (draft stage)	Brooke and Thursby 2000
Quebec, Canada [4]	6.0	water quality criteria for the protection of aquatic life (chronic exposure)	Guay 2000
Quebec, Canada [2]	6.8	interim water quality criteria for the protection of aquatic life (acute exposure)	MEF 1998
U.S.A. [3]	27.9 (1-h average)	ambient aquatic life water quality criteria (draft stage)	Brooke and Thursby 2000
Quebec, Canada [4]	29.0	water quality criteria for the protection of aquatic life (acute exposure)	Guay 2000

[1] Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand 1999

[2] These criteria have recently undergone review, and will likely be updated in 2000 (Guay 2000).

[3] These average concentrations should not be exceeded more than once every three years, on average.

[4] These criteria are still at the draft stage and have not yet been officially approved or implemented (Guay 2000).

Table 6. Freshwater quality guidelines for nonylphenol ethoxylates and ethoxycarboxylates from other jurisdictions.

JURISDICTION	GUIDELINE ($\mu g \cdot L^{-1}$)	CHEMICAL	LEVEL OF PROTECTION	REFERENCE
Denmark	1.0	NP and NPEs	environmental quality standard	Renner 1997
Quebec, Canada [2]	4.0	NP1EC	provisional water quality criteria for the protection of aquatic life (chronic exposure)	Guay 2000
Quebec, Canada [2]	12.0	NP9EO	provisional water quality criteria for the protection of aquatic life (chronic exposure)	Guay 2000
Quebec, Canada [1]	21.0	NPEs	interim water quality criteria for the protection of aquatic life (chronic exposure)	MEF 1998
Quebec, Canada [2]	100.0	NP1EC	provisional water quality criteria for the protection of aquatic life (acute exposure)	Guay 2000
Quebec, Canada [2]	280.0	NP9EO	provisional water quality criteria for the protection of aquatic life (acute exposure)	Guay 2000
Quebec, Canada [1]	470.0	NPEs	interim water quality criteria for the protection of aquatic life (acute exposure)	MEF 1998

[1] These criteria have recently undergone review, and will likely be updated in 2000 (Guay 2000).
[2] These criteria are still at the draft stage and have not yet been officially approved or implemented (Guay 2000).

Table 7. Marine water quality guidelines for nonylphenol from other jurisdictions.

JURISDICTION	GUIDELINE ($\mu g \cdot L^{-1}$)	LEVEL OF PROTECTION	REFERENCE
Australia / New Zealand [1]	1.2	environmental concern level	See footnote
U.S.A.[2]	1.4 (4-d average)	ambient aquatic life water quality criteria (draft stage)	Brooke and Thursby 2000
U.S.A.[2]	6.7 (1-h average)	ambient aquatic life water quality criteria (draft stage)	Brooke and Thursby 2000

[1] Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand 1999

[2] These average concentrations should not be exceeded more than once every three years, on average.

Table 8. Soil and sludge guidelines from other jurisdictions.

JURISDICTION	GUIDELINE ($mg \cdot kg^{-1}$)	CHEMICAL	LEVEL OF PROTECTION	REFERENCE
Denmark	0.01	NP	soil quality criterion	Jensen et al. 1997
Denmark [1]	10	4-NP, NP1EO and NP2EO	guideline for application of sludge to agricultural soils	European Commission 1999
Sweden	50	NP	allowable dry weight concentration in sludge for use as agricultural fertilizer	Bennie et al. 1998

[1] Danish Ministerial Order No. 823, Sept. 16, 1996; guideline takes effect as of June 2000

CHAPTER 5. DEVELOPMENT OF CANADIAN WATER QUALITY GUIDELINES

Canadian Water Quality Guidelines (WQGs) are developed using the methods outlined in "A protocol for the derivation of water quality guidelines for the protection of aquatic life" (CCME 1991; CCME 1999). This protocol was created with the intent that guidelines be protective of all forms of aquatic life at all life stages during an indefinite period of exposure in the water column. A brief summary of the protocol is provided in Annex 2, including a definition of primary data.

Freshwater

Toxicity Data

A large number of studies describing the toxic effects of nonylphenol and its ethoxylates on freshwater aquatic life exist in the scientific literature. A comprehensive list of all freshwater toxicity data for nonylphenol that was consulted has been compiled in Appendix I. Rather than giving an exhaustive review of these studies, a brief description of the toxicity data relevant to the derivation of Canadian Water Quality Guidelines is provided below. This information reflects the data requirements set forth in the protocol (CCME 1991) and is presented by taxonomic groups.

Fish

From the primary toxicity dataset for nonylphenol (Table 9), the most sensitive freshwater fish species is the rainbow trout, *Oncorhynchus mykiss*, with a 91-day LOEC for growth of 10.3 $\mu g \cdot L^{-1}$ (Brooke 1993a). A comparable value of 14 $\mu g \cdot L^{-1}$ was reported as the 33-day LOEC for survival with fathead minnows, *Pimephales promelas* (Ward and Boeri 1991b). The other primary toxicity endpoints are generally an order of magnitude higher. For example, 28-day and 96-hour LOECs for survival have been reported for *Lepomis macrochirus* at 126 and 211 $\mu g \cdot L^{-1}$, respectively, and for *P. promelas* at 193 and 230 $\mu g \cdot L^{-1}$, respectively (Brooke 1993b). Median lethal concentrations (96h LC_{50}s) for *P. promelas* are cited at 128 $\mu g \cdot L^{-1}$ (Brooke 1993a) and 135 $\mu g \cdot L^{-1}$ (Holcombe et al. 1984), and values of 209 and 221 $\mu g \cdot L^{-1}$ are cited for *L. macrochirus* and *O. mykiss*, respectively (Brooke 1993a).

Invertebrates

The most sensitive primary data for a freshwater invertebrate is the 96h LC_{50} of 20.7 $\mu g \cdot L^{-1}$ for *Hyalella azteca* (Brooke 1993a). England and Bussard (1995) also report a 96h LC_{50} of 170 $\mu g \cdot L^{-1}$ and a 96h EC_{50} for immobilization of 150 $\mu g \cdot L^{-1}$ for *H. azteca*. For *Daphnia magna*, concentrations of 84.8, 100, and 215 $\mu g \cdot L^{-1}$ have been reported for a 48h immobilization EC_{50} (Brooke et al. 1993a), a 21-day LC_{50} (Brooke et al. 1993a), and a 21-day LOEC for reproduction (Comber et al. 1993), respectively. Numerous primary datapoints exist for the midge, *Chironomus tentans*, including 14-day EC_{50}s of 95 $\mu g \cdot L^{-1}$ for both total adverse effects and larval growth (England and Bussard 1993), a 14-day LC_{50} of 119 $\mu g \cdot L^{-1}$ (England and Bussard 1993), a 20-day LOEC for survival of 91 $\mu g \cdot L^{-1}$ (Kahl et al. 1997), and a 14-day LOEC for larval growth of 150 $\mu g \cdot L^{-1}$ (England and Bussard 1993). Primary data is only available for two non-arthropod invertebrates. Brooke (1993a) reported 96h LC_{50}s of 342 and 774 $\mu g \cdot L^{-1}$ for the annelid *Lumbriculus variegatus* and the snail *Physella virgata*, respectively.

Plants/Algae

A limited amount of primary data is available for toxicity of nonylphenol to algae and aquatic macrophytes. The macrophyte *Lemna minor* exhibits reduced frond production after 96 hours of exposure to nonylphenol at a concentration of 2080 $\mu g \cdot L^{-1}$ (Brooke 1993a). The green algae *Selenastrum capricornutum* experiences growth reduction after 96 hours of exposure to nonylphenol concentrations as low as 190.0 $\mu g \cdot L^{-1}$, and the corresponding median effective concentration (EC_{50}) is 410 $\mu g \cdot L^{-1}$ (Ward and Boeri 1990a).

Guideline Derivation

Primary data which were selected as acceptable for use in guideline derivation (i.e., that met the screening criteria as described in CCME 1991 and in Annex 2) are shown in Table 9. This dataset is sufficient to meet the minimum requirements for derivation of a full guideline for nonylphenol in fresh water.

According to the protocol, water quality guidelines for a chemical are preferably derived from the chronic study that gives the most sensitive non-lethal lowest-observable-effect level (LOEL) for an ecologically relevant response. The study must have demonstrated a clear dose-response relationship, and the LOEL must be statistically significant..

A chronic toxicity test with rainbow trout, *Oncorhynchus mykiss*, by Brooke (1993a) was selected as the critical study. This study produced the most sensitive acceptable endpoint, a 91d LOEC for reduced growth in rainbow trout at 10.3 $\mu g \cdot L^{-1}$. Rainbow trout were exposed, under flow-through conditions, to two replicates each of five concentrations (ranging from 6 to 114 $\mu g \cdot L^{-1}$) of nonylphenol, plus a control. Each replicate originally contained 100 embryos, and after approximately 45 days, 15 fry from each replicate were randomly chosen for completion of the assay. The total length of the exposure period was 91 days. Temperature, dissolved oxygen, total alkalinity, total hardness, pH, and conductivity were measured regularly through the exposure period and remained within acceptable levels (Brooke 1993a). Water samples from the exposure chambers were collected and analyzed twice per week, throughout the duration of the test. Concentrations of nonylphenol were anaylzed using HPLC. One-way analysis of variance and Dunnett's test were used to determine NOECs and LOECs. In this study, appropriate experimental methods and conditions were used, and all relevant information was reported in the article. Growth is an ecologically relevant response, and the LOEC reported was statistically significant.

Because of possible variations in toxicity among species, among endpoints, and between lab and field exposures, it is recommended that a safety factor be used. Therefore, the value of 10.3 $\mu g \cdot L^{-1}$ (from Brooke 1993a) was multiplied by a safety factor of 0.1 (CCME 1991) to yield 1.03 $\mu g \cdot L^{-1}$. Rounding off, the freshwater guideline value is 1.0 $\mu g \cdot L^{-1}$. The effects diagram in Figure 3 shows the guideline value and the distribution of the primary toxicity data that was used to derive it.

Table 9. Data used in derivation of guideline for toxicity of nonylphenol to freshwater aquatic life.

Species (Common name)	Life cycle stage	Duration	Endpoint	Effect	Concentration ($\mu g \cdot L^{-1}$)	Reference
PRIMARY DATA						
Algae/Macrophytes						
Lemna minor (duckweed)	2-frond stage	96h	LOEC	frond production	2080	Brooke 1993a
Selenastrum capricornutum (algae)	4-7 days	96h	LOEC	growth (cell counts)	190	Ward and Boeri 1990a
	4-7 days	96h	EC_{50}	growth (cell count)	410	Ward and Boeri 1990a
Invertebrates						
Chironomus tentans (midge)	larva	20d	LOEC	survival	91	Kahl et al. 1997
	2nd instar larva	14d	EC_{50}	lethargy, paleness, reduced size	95	England and Bussard 1993
	2nd instar larva	14d	EC_{50}	larval growth	95	England and Bussard 1993
	2nd instar larva	14d	LC_{50}	mortality	119	England and Bussard 1993
	2nd instar larva	14d	LOEC	larval growth	150	England and Bussard 1993
Daphnia magna (water flea)	neonate	48h	EC_{50}	immobilization	84.8	Brooke 1993a
	neonate	21d	LC_{50}	mortality	100	Comber et al. 1993
	neonate	21d	LOEC	reproduction	215	Brooke 1993a
Hyalella azteca (amphipod)	juvenile	96h	LC_{50}	mortality	20.7	Brooke 1993a
	juvenile	96h	EC_{50}	lethargy/ immobility	150	England and Bussard 1995b
	juvenile	96h	LC_{50}	mortality	170	England and Bussard 1995b
Lumbriculus variegatus (annelid)	adult	96h	LC_{50}	mortality	342	Brooke 1993a
Physella virgata (snail)	adult	96h	LC_{50}	mortality	774	Brooke 1993a
Fish						
Lepomis macrochirus (bluegill sunfish)	11-13 weeks	28d	LOEC	survival	126	Brooke 1993b
	juvenile	96h	LC_{50}	mortality	209	Brooke 1993a
	13-15 weeks	96h	LOEC	survival	211	Brooke 1993b
Oncorhynchus mykiss (rainbow trout)	embryo	91d	LOEC	growth	10.3	Brooke 1993a
	45 days	96h	LC_{50}	mortality	221	Brooke 1993a

Table 9 (continued). Data used in derivation of guideline for toxicity of nonylphenol to freshwater aquatic life.

Species (Common name)	Life cycle stage	Duration	Endpoint	Effect	Concentration ($\mu g \cdot L^{-1}$)	Reference
Pimephales promelas (Fathead minnow)	embryo	33d	LOEC	survival	14	Ward and Boeri 1991b
	25-35 days	96h	LC_{50}	mortality	128	Brooke 1993a
	31-35 days	96h	LC_{50}	mortality	135	Holcombe et al. 1984
	4 weeks	28d	LOEC	survival	193	Brooke 1993b
	4 weeks	96h	LOEC	survival	230	Brooke 1993b

LOEC = lowest observed effect concentration
LC_{50} = median lethal concentration
EC_{50} = median effective concentration

Note: The critical study used in the guideline derivation is shaded.

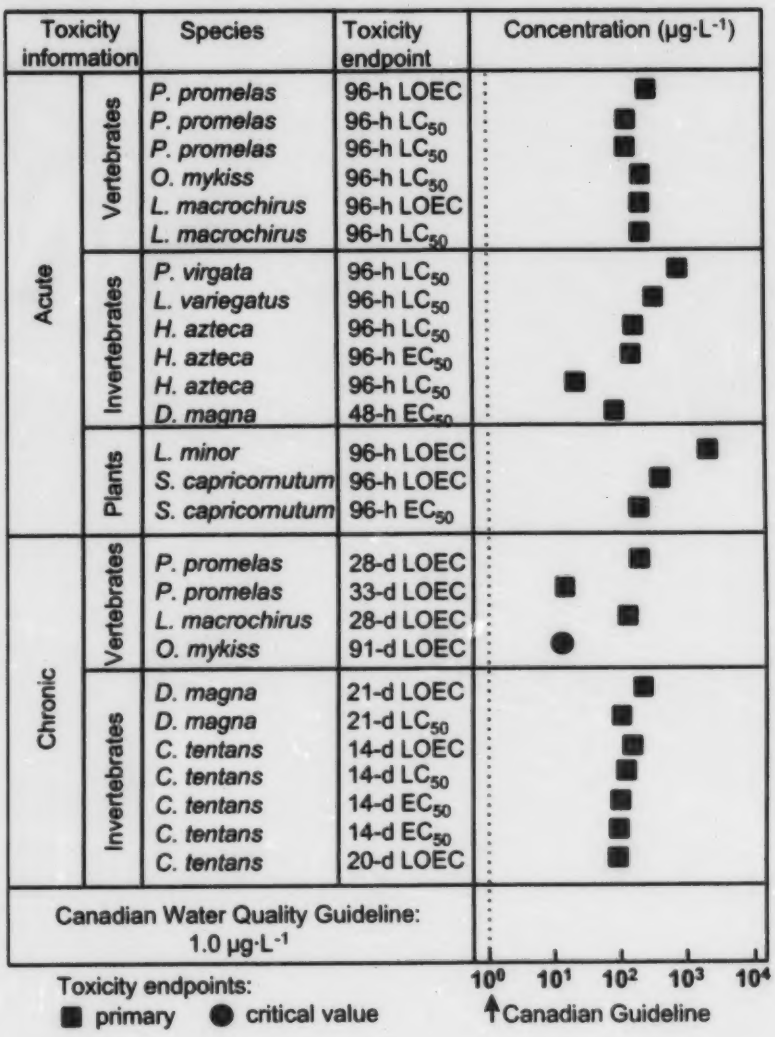


Figure 3. Select freshwater toxicity data for nonylphenol.

Marine/Estuarine Water

Toxicity Data

A large number of studies describing the toxic effects of nonylphenol and its ethoxylates on marine aquatic life exist in the scientific literature. A comprehensive list of all marine toxicity data for nonylphenol that was consulted has been compiled in Appendix II. Rather than giving an exhaustive review of these studies, a brief description of the toxicity data relevant to the derivation of Canadian Water Quality Guidelines is provided below. This information reflects the data requirements set forth in the protocol (CCME 1991) and is presented by taxonomic groups.

Fish

From the nonylphenol toxicity dataset, three acceptable (secondary) chronic toxicity studies were available for marine fish. A 21-day LOEC of 24.5 $\mu g \cdot L^{-1}$ was reported for increased hepatosomatic index in flounder, *Platichthys flesus* (Allen et al. 1999). A 21-day LOEC of 100 $\mu g \cdot L^{-1}$ was reported for increased plasma concentrations of vitellogenin in eelpout, *Zoarces viviparus* (Korsgaard and Pedersen 1998). A 10-day LOEC of 2203 $\mu g \cdot L^{-1}$ for the induction of sublethal abnormalities in embryos of the killifish, *Fundulus heteroclitus*, was also reported (Kelly and Di Giulio 2000). Six acceptable acute toxicity endpoints have been reported for marine fish. The 96h LC_{50} for sheepshead minnow, *Cyprinodon variegatus*, has been estimated at 310.0 $\mu g \cdot L^{-1}$ (Ward and Boeri 1990c) and 142.0 $\mu g \cdot L^{-1}$ (Lussier et al. 2000). For inland silversides, *Menidia beryllina*, a 96h LC_{50} of 70.0 $\mu g \cdot L^{-1}$ has been cited (Lussier et al. 2000). A 96h LC_{50} of 17 $\mu g \cdot L^{-1}$ has been measured in winter flounder, *Pleuronectes americanus* (Lussier et al. 2000). In *F. heteroclitus*, 96h LC_{50}s have been reported for embryos and for 2-week larvae at 5441 and 209 $\mu g \cdot L^{-1}$, respectively (Kelly and Di Giulio 2000).

Invertebrates

The lowest reported endpoint for a marine invertebrate came from a secondary study by Billinghurst et al. (1998) in which the 48h LOEC for reduced settlement in larvae of the barnacle *Balanus amphitrite* was 0.06 $\mu g \cdot L^{-1}$. Several studies indicate that the mysid *Americamysis bahia* (recently renamed from *Mysidopsis bahia*) is also a sensitive marine invertebrate. Ward and Boeri (1991a) reported reductions in the growth of *A. bahia* after a 28-day exposure to nonylphenol concentrations as low as 6.7 $\mu g \cdot L^{-1}$ and reduced reproduction and survival were both observed at concentrations as low as 9.1 $\mu g \cdot L^{-1}$. Ward and Boeri (1990b) also reported a 96h LC_{50} for *A. bahia* of 43.0 $\mu g \cdot L^{-1}$, and Lussier et al. (2000) reported a 96h LC_{50} of 60.6 $\mu g \cdot L^{-1}$ for this same species. Slightly higher 96h LC_{50}s of 59.4, 61.6, 71.0, and >195 $\mu g \cdot L^{-1}$ are reported for the grass shrimp *Paleomonetes vulgaris*, the amphipod *Leptocheirus plumulosus*, the lobster *Homarus americanus*, and the mudcrab *Dyspanopeus sayii*, respectively (Lussier et al. 2000). Lussier et al. (2000) also reported a 48h EC_{50} of 37.9 $\mu g \cdot L^{-1}$ for inhibition of fertilization in the coot clam *Mulinia lateralis*. From a 53-day study on the copepod *Tisbe battagliai*, a LOEC of 41.0 $\mu g \cdot L^{-1}$ has been reported for egg production, offspring survival, and intrinsic rate of increase (Bechmann 1999). For this same species, the 96h LC_{50}s for nauplii and for adults are 31-62 $\mu g \cdot L^{-1}$ and 500-1000 $\mu g \cdot L^{-1}$, respectively (Bechmann 1999). For larvae of the Pacific oyster, *Crassostrea gigas*, exposure to 100 $\mu g \cdot L^{-1}$ resulted in both a 48h LOEC for various deformities, and a 72h LOEC for delayed development (Nice et al. 2000).

Algae

The marine alga *Skeletonema costatum* has a 96 hour EC_{50} for growth inhibition of 27.0 $\mu g \cdot L^{-1}$ (Ward and Boeri 1990d). This is the only acceptable toxicity study available for marine algae or plants.

Guideline Derivation

Primary data that were selected as acceptable for use in guideline derivation (i.e., that met the screening criteria as described in CCME 1991 and in Annex 2) are shown in Table 10. These data are insufficient to meet the minimum requirements for derivation of a full guideline for the protection of aquatic marine life. Three additional primary studies are needed for a full guideline. Of these studies, two must include chronic endpoints for two different temperate, marine fish species. The third study must have a chronic endpoint for a temperate invertebrate that belongs to a class other than the Crustacea.

When the minimum data requirements for derivation of a full guideline cannot be met, it may still be possible to derive an interim guideline. Secondary studies, as well as primary studies, may be used to meet the minimum requirements for an interim guideline. Studies which were considered of secondary quality, and therefore could be used to meet the minimum data requirements for an interim guideline, are also listed in Table 10. Using both the primary and secondary studies, there are sufficient data to meet the minimum requirements for deriving an interim guideline for nonylphenol.

As for freshwater, marine water quality guidelines are preferably derived from the chronic study that gives the most sensitive nonlethal LOEL (CCME 1991). The LOEL is then multiplied by a safety factor of 0.1 to arrive at the final guideline value.

The LOEC for reduced settlement in barnacles, *Balanus amphitrite*, was the most sensitive endpoint reported (48h LOEC at 0.06 $\mu g \cdot L^{-1}$; Billinghurst et al. 1998). However, this study was of secondary quality, involved a short exposure time, and used an unconventional endpoint. Also, this LOEC value was two orders of magnitude lower than any other endpoints reported in the literature. This suggests that either larval settlement is much more sensitive than other endpoints, or that this particular LOEC is an outlier that is not reproducible. Unfortunately, there have been no other studies conducted on barnacle larval settlement with exposure to nonylphenol with which to compare these results. The LOECs reported for larval settlement of *Balanus amphitrite* exposed to cadmium and phenol (Wu et al. 1997) are higher than the LOECs for many other endpoints that have been reported for these substances in marine aquatic life (see CCME 1999). This suggests that larval settlement is not a particularly sensitive endpoint. Due to the suspicion cast by these various sources of uncertainty, confidence in the study by Billinghurst et al. (1998) was not high enough to base the guideline value on this LOEC.

The chronic assay with the mysid, *Americamysis bahia*, by Ward and Boeri (1991a) was selected as the critical study. This study reported a 28-day LOEC for reduced growth at 6.7 $\mu g \cdot L^{-1}$ (Ward and Boeri 1991a). The study was evaluated as being of primary quality because appropriate experimental methods and conditions were used, and all relevant information was reported. The test protocol used was based on U.S. EPA test guidelines. The study involved exposure of mysids, under flow-through conditions, to five concentrations of nonylphenol (ranging from 3.9 to 21 $\mu g \cdot L^{-1}$), plus a dilution water control and solvent control (acetone). Eight replicates of each test concentration were used, with five mysids per replicate. The total length of the exposure period was 28 days. Temperature, dissolved oxygen, pH, and salinity were measured and recorded daily throughout the exposure period and remained within acceptable levels. Water samples from the test vessels were sampled weekly, with nonylphenol concentrations anaylzed by HPLC with fluorescence detection. One-way analysis of variance and Dunnett's test were used to test for treatment effects. Growth is an ecologically relevant response, and the LOEC reported was statistically significant.

Therefore, the LOEC value of 6.7 $\mu g \cdot L^{-1}$ (from Ward and Boeri 1991a) was multiplied by a safety factor of 0.1 (CCME 1991), and rounded off, to obtain the interim guideline of 0.7 $\mu g \cdot L^{-1}$ for the protection of marine life against the adverse effects of nonylphenol. The effects diagram in Figure 4 shows the interim guideline value and the distribution of the toxicity data that was used to derive it.

Table 10. Data used in derivation of guideline for toxicity of nonylphenol to marine aquatic life.

Species (Common name) **PRIMARY DATA**	Life cycle stage	Duration	Endpoint	Effect	Concentration ($\mu g \cdot L^{-1}$)	Reference
Algae						
Skeletonema costatum (algae)	NR	96h	EC_{50}	growth inhibition (cell count)	27	Ward and Boeri 1990d
Invertebrates						
Americamysis bahia (mysid)	neonates	28d	LOEC	mean total length	6.7	Ward and Boeri 1991a
	neonates	28d	LOEC	survival	9.1	Ward and Boeri 1991a
	neonates	28d	LOEC	number of young per surviving female	9.1	Ward and Boeri 1991a
	neonates	96h	LC_{50}	mortality	43	Ward and Boeri 1990b
	post-larvae 1d	96h	LC_{50}	mortality	60.6	Lussier et al. 2000
Leptocheirus plumulosus (amphipod)	adult	96h	LC_{50}	mortality	61.6	Lussier et al. 2000
Paleomonetes vulgaris (grass shrimp)	2d larvae	96h	LC_{50}	mortality	59.4	Lussier et al. 2000
Tisbe battagliai (copepod)	nauplii	53d	LOEC	egg production, offspring survival, intrinsic rate of increase	41	Bechmann 1999
Fish						
Cyprinodon variegatus (sheepshead minnow)	juveniles	96h	LC_{50}	mortality	142	Lussier et al. 2000
	juveniles	96h	LC_{50}	mortality; lethargy, bloating, loss of equilibrium	310	Ward and Boeri 1990c
Menidia beryllina (inland silversides)	juveniles	96h	LC_{50}	mortality	70	Lussier et al. 2000

Table 10 (continued). Data used in derivation of guideline for toxicity of nonylphenol to marine aquatic life.

Species (Common name)	Life cycle stage	Duration	Endpoint	Effect	Concentration ($\mu g \cdot L^{-1}$)	Reference
SECONDARY DATA						
Invertebrates						
Balanus amphitrite (barnacle)	cyprids (larvae)	48h	LOEC	reduced settlement	0.06	Billinghurst et al. 1998
Crassostrea gigas (Pacific oyster)	larvae	48h	LOEC	deformity	100	Nice et al. 2000
	larvae	72h	LOEC	delayed development	100	Nice et al. 2000
Dyspanopeus sayii (mudcrab)	4th and 5th stage zoea	96h	LC_{50}	mortality	> 195	Lussier et al. 2000
Homarus americanus (American lobster)	first stage zoea	96h	LC_{50}	mortality	71	Lussier et al. 2000
Mulinia lateralis (Coot clam)	embryo-larval	48h	EC_{50}	inhibition of fertilization	37.9	Lussier et al. 2000
Tisbe battagliai (copepod)	<1 day nauplii	96h	LC_{50}	mortality	31-62	Bechmann 1999
	adult	96h	LC_{50}	mortality	500-1000	Bechmann 1999
Fish						
Fundulus heteroclitus (killifish)	2 wk larvae	96h	LC_{50}	mortality	209	Kelly and Di Giulio 2000
	embryos	10d	LOEC	sublethal abnormalities	2203	Kelly and Di Giulio 2000
	embryos	96h	LC_{50}	mortality	5441	Kelly and Di Giulio 2000
Platichthys flesus (flounder)	adults	21d	LOEC	increased hepato-somatic index	24.5	Allen et al. 1999
Pleuronectes americanus (winter flounder)	2d larvae	96h	LC_{50}	mortality	17	Lussier et al. 2000
Zoarces viviparus (eelpout)	pregnant females	21d	LOEC	increased plasma levels of vitellogenin	100	Korsgaard and Pedersen 1998

LOEC = lowest observed effect concentration
LC_{50} = median lethal concentration
EC_{50} = median effective concentration
NR = not reported
Note: The critical study used in the guideline derivation is shaded.

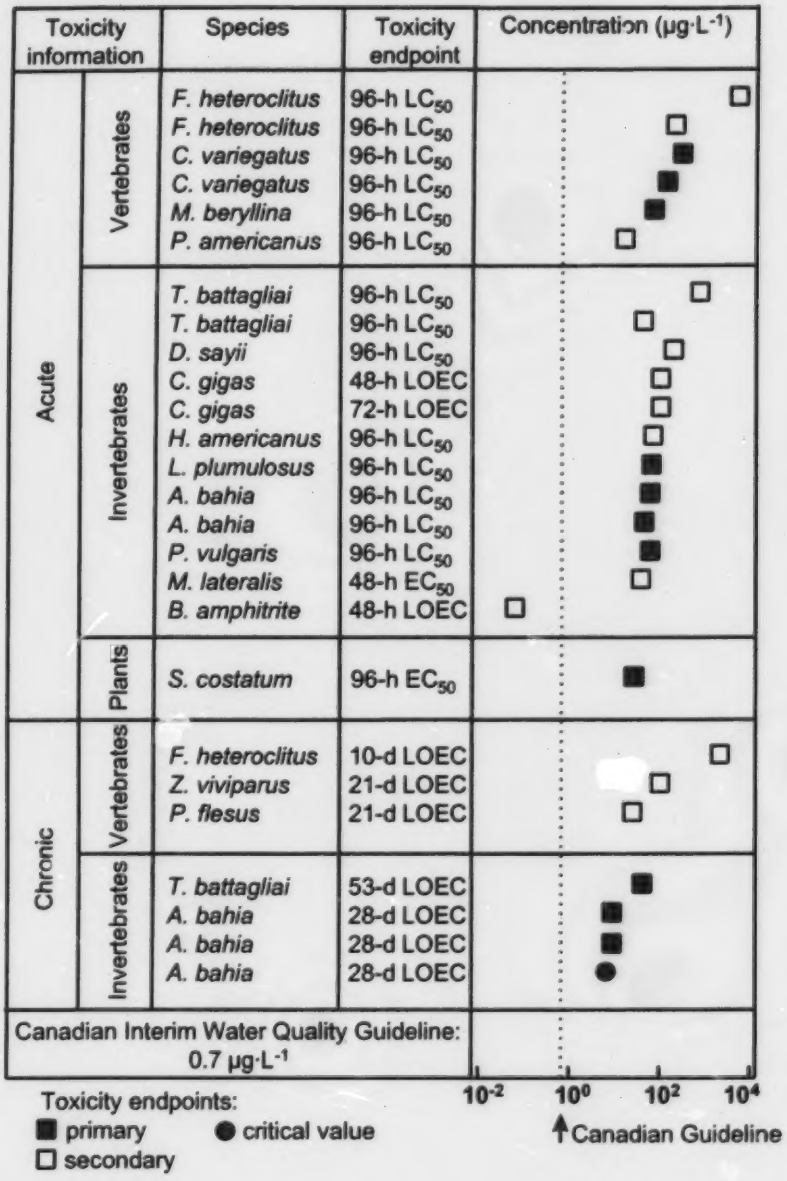


Figure 4. Select marine toxicity data for nonylphenol.

Recommended Canadian Water Quality Guidelines

Guideline for the Protection of Freshwater Life

A full guideline of 1.0 $\mu g \cdot L^{-1}$, expressed in nonylphenol toxic equivalency units (NP TEQs), is recommended for the protection of aquatic freshwater life from the adverse effects of nonylphenol and its ethoxylates.

There were sufficient toxicity data available to derive a full freshwater guideline. This guideline should be applied to the total concentration (in NP TEQs) of all nonylphenolic substances occurring in a water sample. The total concentration should be calculated using the nonylphenol toxic equivalency factors provided in Chapter 3 (Table 4). Further guidance and recommendations for the implementation of this guideline are provided in Chapter 9.

The freshwater guideline for nonylphenol and its ethoxylates proposed in this document is the same as the environmental quality standard of 1.0 $\mu g \cdot L^{-1}$ that exists in Denmark (see Table 5). It is lower than both the acute and chronic U.S. criteria for nonylphenol (at 27.9 $\mu g \cdot L^{-1}$ and 5.9 $\mu g \cdot L^{-1}$, respectively), but is higher than the environmental concern level of 0.2 $\mu g \cdot L^{-1}$ proposed by Australia/New Zealand (see Table 5). It is also higher than Quebec's interim water quality criteria for chronic exposure of 0.3 $\mu g \cdot L^{-1}$, although recommendations have been made to increase this value to 6.0 $\mu g \cdot L^{-1}$ (Guay 2000). The Ontario provincial objective, at 0.04 $\mu g \cdot L^{-1}$, is more than an order of magnitude lower than the Canadian water quality guideline proposed here. It should be noted that the Ontario objective was calculated using an extremely conservative safety factor because at the time of its development, in 1991, there were much fewer toxicity data available.

Guideline for the Protection of Marine Life

An interim guideline of 0.7 $\mu g \cdot L^{-1}$, expressed in NP TEQs, is recommended for the protection of aquatic marine life from the adverse effects of nonylphenol and its ethoxylates.

This guideline is expressed in nonylphenol toxic equivalency (NP TEQ) units and may be applied to mixtures of nonylphenol and nonylphenol ethoxylates. The total concentration of nonylphenolic substances in a water sample (in NP TEQs) should be calculated using the nonylphenol toxic equivalency factors provided in Chapter 3 (Table 4). Further guidance and recommendations for the implementation of this guideline are provided in Chapter 9.

The guideline proposed here is lower than both the environmental concern level for nonylphenol of Australia/New Zealand, at 1.2 $\mu g \cdot L^{-1}$, and the U.S. acute and chronic criteria of 6.7 $\mu g \cdot L^{-1}$ and 1.4 $\mu g \cdot L^{-1}$, respectively (see Table 7).

CHAPTER 6. DEVELOPMENT OF CANADIAN SEDIMENT QUALITY GUIDELINES

Canadian Sediment Quality Guidelines are developed using the methods outlined in "Protocol for the derivation of Canadian sediment quality guidelines for the protection of aquatic life" (CCME 1995, 1999). This protocol was created with the intent that guidelines be protective of all forms of aquatic life and all aspects of their aquatic life cycles during an indefinite period of exposure to substances associated with bed sediments.

Toxicity Data

Data on the toxicity of nonylphenol in sediments to aquatic life were only available for invertebrates and amphibians, and were quite limited. These data are also presented in Appendix III.

Invertebrates

Toxicity of nonylphenol in sediment has been determined for a single freshwater invertebrate species, the midge, *Chironomus tentans* (England and Bussard 1993). The study used spiked-sediment toxicity testing to assess both lethal and sublethal effects of nonylphenol on the midge larvae over a 14 day exposure period. Acceptable spiking procedures were used and a standard test protocol was followed. For both mortality and growth, NOEC values were reported at 20.1 $mg \cdot kg^{-1}$ dry weight (England and Bussard 1993). A LOEC of 34.2 $mg \cdot kg^{-1}$ (dry weight) was observed for both mortality and growth, with 31% mortality and a growth reduction of 27% occurring at that concentration.

Two studies have examined the toxicity of nonylphenol to marine invertebrates, both through spiked-sediment toxicity testing. One study evaluated nonylphenol lethality to the amphipod *Ampelisca abdita* (Fay et al. 2000). Standard sediment spiking and test procedures were followed. A ten day LC_{50} of 98.7 $mg \cdot kg^{-1}$ was observed, with 100% mortality occurring at a concentration of 243 $mg \cdot kg^{-1}$. The other study evaluated sub-lethal effects of nonylphenol on the polychaete *Capitella* sp. I over a 78 day exposure period (Hansen et al. 1999). Sub-lethal endpoints examined included time to first reproduction, time between reproductive events, number of broods per reproductive individual, number of eggs per individual and per reproductive individual, population growth rate, and size at maturity. For all of these endpoints, the NOEC was 52 $mg \cdot kg^{-1}$ and the LOEC was 174 $mg \cdot kg^{-1}$ (Hansen et al. 1999). The results of this study, however, should be treated with caution because standard sediment spiking procedures were not followed.

Amphibians

Ward and Boeri (1992) used a 30 day spiked-sediment toxicity test to examine toxicity of nonylphenol to tadpoles of the bullfrog, *Rana catesbiana*. Standard sediment spiking and test procedures were followed. Reported endpoints for mortality included a NOEC of 155 $mg \cdot kg^{-1}$ and a LOEC of 390 $mg \cdot kg^{-1}$ with a calculated LC_{50} of 260 $mg \cdot kg^{-1}$. The authors also examined sub-lethal endpoints such as growth, lethargy, haemorrhaging, and occurrence of tail lesions. For all of these sub-lethal effects, the NOECs were 155 $mg \cdot kg^{-1}$, the LOECs were 390 $mg \cdot kg^{-1}$, and EC_{50}s were calculated at 220 $mg \cdot kg^{-1}$ (Ward and Boeri 1992).

Protocol Approaches

The protocol relies on the modified National Status and Trends Program (NSTP) approach and the Spiked Sediment Toxicity Test (SSTT) approach. If sufficient information exists to support both methods, a full sediment quality guideline may be recommended. If information is only available to support one of the approaches, then an interim sediment quality guideline is recommended. In cases where there is insufficient information to derive interim guidelines using either the modified NSTP approach or the SSTT approach, provisional guidelines may be

recommended by adopting guidelines from other jurisdictions. Further discussion of these three approaches is provided in Annex 3 of this document. At present, insufficient information exists to derive sediment quality guidelines for nonylphenol using any of the three approaches described in the protocol.

The protocol (CCME 1995) does not provide guidance on other possible options if there is insufficient data to meet the minimum data requirements for the NSTP and SSTT approaches, and if there are no acceptable sediment quality guidelines from other jurisdictions that can be adopted. One possibility would be to not derive a guideline at all. While acknowledging the need for toxicity data of the highest quality, we believe that guidelines based on the best available data and protocol approaches are still more practical, scientifically defensible, and protective of the environment than the absence of guidelines, or than guidelines that are not risk-based. Therefore, we are recommending the use of an equilibrium partitioning approach for the development of provisional Canadian interim sediment quality guidelines for nonylphenol and its ethoxylates.

Equilibrium Partitioning Approach

The water/sediment equilibrium partitioning approach is used in the United States for development of sediment quality guidelines (MacDonald et al. 1992) and is also supported by the Ontario Ministry of the Environment (OMOE 1993). This approach establishes a guideline based on the concentration of a substance in sediments that ensures that the concentration in the interstitial water does not exceed the water quality guideline for that chemical at equilibrium (USEPA 1992). It is based on the assumption that continuous equilibrium exchange of a contaminant between sediment and interstitial water occurs (MacDonald et al. 1992). For non-polar organic substances, such as nonylphenol, it is known that concentrations in the interstitial water are dependent on concentrations in the sediment phase with the partitioning largely controlled by the total organic carbon (TOC) content of the sediment (MacDonald et al 1992). This appears to be true for NP and NPEs. Studies have shown that organic content is an important determining factor in the adsorption of at least the short-chain NPEs to sediments (Urano 1984; John et al. 2000). Johnson et al. (1998) found a positive relationship between amounts of octylphenol sorbed to sediments and the amount of TOC in the sediments; one would expect NP to behave similarly. Through spiked-sediment bioaccumulation tests with benthic amphipods, Hecht et al. (2000) found that higher concentrations of NP moved into the pore water of those sediments containing lower TOC concentrations. Based on similar studies, Gunnarsson et al. (2000) also found that pore water appears to be the major route of exposure to NP for benthic amphipods, and they concluded that the equilibrium partitioning theory is valid for nonylphenol.

As the protocol for the development of Canadian sediment quality guidelines (CCME 1995) allows for the adoption of guidelines from other jurisdictions that were derived using the equilibrium partitioning approach, it seems reasonable, and in keeping with the spirit of the protocol, that this approach could be used to derive sediment quality guidelines based on the Canadian water quality guidelines for nonylphenol and its ethoxylates. Sediment quality assessment values based on partitioning are less ecologically relevant and scientifically defensible than effect-based guidelines that are derived from direct sediment exposure studies (CCME 1995). The equilibrium partitioning method assumes that sediment-dwelling organisms and water column organisms are equally sensitive to nonylphenol and that the concentration of NP in sediment, interstitial water, and benthic organisms is at thermodynamic equilibrium. However, because there are insufficient data available for derivation of sediment quality guideline values by other methods, the equilibrium partitioning approach will be used to develop provisional ISQGs.

Guideline Derivation

Using the equilibrium partitioning approach, provisional ISQGs were derived from the Canadian water quality guidelines for nonylphenol and its ethoxylates, developed in Chapter 5, using the following equation (Di Toro et al. 1991; MacDonald et al. 1992):

$$ISQG = WQG \cdot K_p \qquad \text{Equation 1}$$

Where:

WQG = water quality guideline (in $\mu g \cdot L^{-1}$);
K_p = sediment/water partition coefficient (in $L \cdot kg^{-1}$)

For non-polar organic contaminants such as nonylphenol, the organic carbon content of the sediment is a major factor controlling the partitioning between water and sediment (MacDonald et al. 1992). The sediment/water partition coefficient can therefore be related to the organic carbon/water partition coefficient by the following equation (Di Toro et al. 1991):

$$K_p = K_{oc} \cdot f_{oc} \qquad \text{Equation 2}$$

Where:

K_{oc} = organic carbon/water partition coefficient (in $L \cdot kg_{oc}^{-1}$);
f_{oc} = fraction of organic carbon

Therefore, by substituting Equation 2 into Equation 1, the ISQG may be calculated as:

$$ISQG = WQG \cdot K_{oc} \cdot f_{oc} \qquad \text{Equation 3}$$

Sekela et al. (1999) estimated that the log K_{oc} for nonylphenol ranges from 4.7 to 5.6. Taking the anti-log of these two values, and then calculating the geometric mean, yields a K_{oc} of 141 254 $L \cdot kg_{oc}^{-1}$. The freshwater Canadian WQG for nonylphenol, derived herein, is 1.0 $\mu g \cdot L^{-1}$. Substituting these values into Equation 3 yielded a freshwater ISQG of 141 mg NP per kg of organic carbon. This value was adjusted in order to express the guideline as dry weight of sediment, so that the guideline would be consistent with other Canadian SQGs. Therefore, by normalizing to 1% TOC (i.e., f_{oc} = 0.01), the freshwater ISQG of 1.4 $mg \cdot kg^{-1}$ dry weight was derived. It is recommended that the guideline be adjusted, as described in Chapter 9, based on local levels of TOC to obtain a site-specific objective.

Similarly for marine sediments, an ISQG was derived by substituting into Equation 3 the K_{oc} of 141 254 $L \cdot kg_{oc}^{-1}$, and the marine Canadian WQG of 0.7 $\mu g \cdot L^{-1}$. This resulted in an ISQG of 99 mg NP per kg of organic carbon. The value was expressed as dry weight of sediment by normalizing to 1% TOC, to give a marine ISQG of 1.0 $mg \cdot kg^{-1}$ dry weight. Again, it is recommended that the guideline be adjusted, as described in Chapter 9, based on local levels of TOC to obtain a site-specific objective.

Comparing these guideline values with the available sediment toxicity data, it would appear that they are likely to be protective of most sediment-dwelling organisms. The lowest effect concentrations observed in freshwater and marine sediment toxicity tests with nonylphenol were 34.2 $mg \cdot kg^{-1}$ and 174 $mg \cdot kg^{-1}$, respectively. The guidelines are lower than these effect concentrations by more than a factor of 20, allowing for a large margin of safety.

It should be noted that because these guidelines were derived using an equilibrium partitioning approach, they represent sediment concentrations that will be protective of sediment-dwelling biota when the sediment and interstitial water are in equilibrium. Their use requires the assumptions that there are no changes in the thermodynamics of the system (i.e., constant temperature and pressure with no input/output of energy or mass), and that there has been sufficient time for the NP and/or NPEs to distribute throughout the system (Spacie et al. 1995). It is possible that non-equilibrium conditions could exist in some cases, such as immediately

following a spill, in river channels where the sediments are unstable, or in active dredge disposal areas (NYSDEC 1999). The USEPA SAB (1992) cautions against the use of guidelines based on equilibrium partitioning in areas of rapid sediment deposition or erosion. Where partitioning of nonylphenolic compounds between the sediment and interstitial water is not in equilibrium, it is possible that the sediment guidelines may not be protective against adverse effects to benthos.

Recommended Canadian Sediment Quality Guidelines

Freshwater Sediments

Insufficient toxicological data exist for freshwater sediments to develop a freshwater TEL and PEL using the modified NSTP approach, or to calculate a freshwater SSTT value using the SSTT approach. There are also no sediment quality guidelines that currently exist in other jurisdictions that could be adopted. Therefore, a provisional freshwater ISQG was calculated from the Canadian freshwater WQG for nonylphenol proposed in this document using the equilibrium partitioning approach.

A provisional interim sediment quality guideline of 1.4 mg·kg^{-1} dry weight, expressed in nonylphenol toxic equivalent units (NP TEQs) and normalized to 1% TOC, is recommended for the protection of freshwater life from the adverse effects of nonylphenol and its ethoxylates.

This guideline is recommended for concentrations of nonylphenolic substances in freshwater bulk surficial sediments (i.e., top 5 cm). The guideline is expressed in nonylphenol toxic equivalency (NP TEQ) units and may be applied to mixtures of nonylphenol and nonylphenol ethoxylates. The total concentration of nonylphenolic substances in a sediment sample (in NP TEQs) should be calculated using the nonylphenol toxic equivalency factors provided in Chapter 3 (Table 4). The guideline should be adjusted for site-specific levels of total organic carbon. Further guidance and recommendations for the implementation of this guideline are provided in Chapter 9.

The distribution of the toxicity data for nonylphenol in freshwater sediments, as compared with the provisional ISQG is shown in Figure 5. There are no effects concentrations below the provisional freshwater ISQG; therefore, it is thought to be protective of aquatic organisms.

Marine Sediments

As was the case for freshwater sediments, insufficient toxicological data exist for marine sediments to develop a marine TEL and PEL using the modified NSTP approach, or to calculate a freshwater SSTT value using the SSTT approach. Also, there are no marine sediment quality guidelines that currently exist for nonylphenol in any other jurisdictions. Therefore, a provisional marine ISQG was calculated from the Canadian marine WQG for nonylphenol proposed in this document by using the equilibrium partitioning approach.

A provisional interim sediment quality guideline of 1.0 mg·kg^{-1} dry weight, expressed in NP TEQs and normalized for 1% TOC, is recommended for the protection of marine life from the adverse effects of nonylphenol and its ethoxylates.

This guideline is recommended for concentrations of nonylphenolic substances in marine bulk surficial sediments (i.e., top 5 cm). The guideline is expressed in toxic equivalency (TEQ) units and may be applied to mixtures of nonylphenol and nonylphenol ethoxylates. The total concentration of nonylphenolic substances in a sediment sample (in NP TEQs) should be calculated using the nonylphenol toxic equivalency factors provided in Chapter 3 (Table 4). The guideline should be adjusted based on site-specific levels of total organic carbon. Further guidance and recommendations for the implementation of this guideline are provided in Chapter 9.

The distribution of the toxicity data for nonylphenol in marine sediments, as compared with the provisional ISQG is shown in Figure 6. There are no effects concentrations below the provisional marine ISQG; therefore, it is thought to be protective of aquatic organisms.

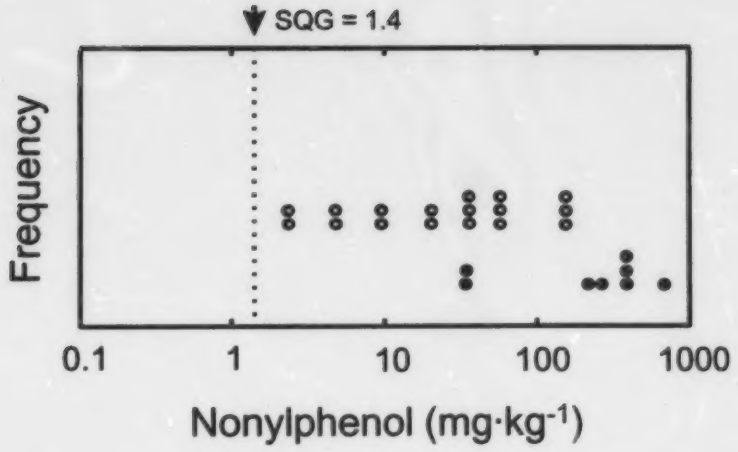


Figure 5. Distribution of nonylphenol concentrations in freshwater sediments that are associated with adverse biological effects (●) and no adverse effects (○).
The provisionally recommended Canadian freshwater interim sediment quality guideline (SQG) is indicated by the dotted line.

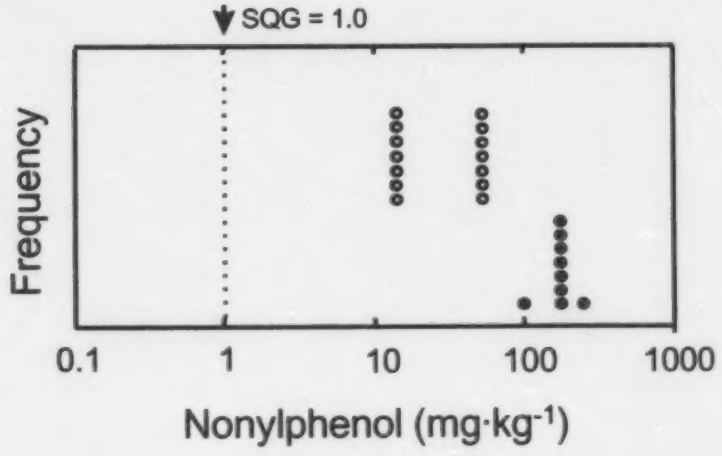


Figure 6. Distribution of nonylphenol concentrations in marine and estuarine sediments that are associated with adverse biological effects (●) and no adverse effects (○).
The provisionally recommended Canadian marine interim sediment quality guideline (SQG) is indicated by the dotted line.

CHAPTER 7. DEVELOPMENT OF CANADIAN SOIL QUALITY GUIDELINES

Canadian soil quality guidelines are developed using the methods outlined in "A protocol for the derivation of environmental and human health soil quality guidelines" (CCME 1996). This protocol was created with the intent that guidelines be protective of terrestrial biota and ensure a healthy functioning ecosystem capable of sustaining current and possible future uses of a site by ecological receptors and humans. Human health guidelines have not been developed for nonylphenol, therefore, this chapter discusses only the environmental health component of the Canadian soil quality guidelines for nonylphenol. According to the protocol, soil quality guidelines for each chemical are developed for four different land use categories: agricultural, residential/parkland, commercial, and industrial. A flow chart illustrating the steps that were taken in developing the environmental soil quality guidelines for nonylphenol is provided in Figure 7. The primary procedure for derivation of environmental soil quality guidelines is through the use of direct soil contact toxicological data. A second procedure based on soil and food ingestion is also applied in the case of agricultural land uses (if sufficient data is available), with adoption of the lowest of the two values as the guideline for agricultural land use.

Toxicity Data

The first stage in development of environmental soil quality guidelines is the compilation of toxicity data that is evaluated based on the acceptability of experimental design, laboratory procedures, and documentation of relevant experimental conditions and parameters. Studies that provide high quality data are classified as "accepted" and this toxicity data may be used in deriving the guideline. Studies in which key information is missing, or in which experimental methods or conditions are questionable, are classified as "consulted" and are not used in the derivation of the guideline value, though their results may be taken into consideration. A comprehensive list of all soil toxicity data that was consulted has been compiled in Appendix IV. Studies were excluded from use because of one or more of the following reasons:

1. the test was not conducted using soil or artificial soil (e.g., conducted in nutrient media or on agar)
2. the test soil was amended with sewage sludge or a mixture of toxicants, such that effects could not be conclusively attributed to nonylphenol
3. important details of the experimental procedure were not described (e.g., number of NP concentrations tested, analytical methodology used, etc.)

The primary data selected as acceptable for use in guideline derivation are shown in Table 11. Effective concentration data in this table were considered to be biologically significant in addition to statistically significant.

A brief description of the toxicity data relevant to the derivation of Canadian Soil Quality Guidelines is provided below. This information reflects the data requirements set forth in the protocol (CCME 1996) and is presented by taxonomic groups.

Microorganisms

A number of studies have looked at the toxic effects of nonylphenol on microorganisms in artificial nutrient media, but only toxicity tests conducted with soil are described here. The effects of nonylphenol on the respiration of soil microorganisms, as measured through CO_2 evolution, have been studied by two sets of researchers. In a 40 day test, Trocmé et al. (1988) observed no effect of CO_2 evolution when nonylphenol was present in the soil at a concentration of 100 $mg \cdot kg^{-1}$, but at 1000 $mg \cdot kg^{-1}$ there was a significant reduction in CO_2 evolution of 64%. In a three month assay, Kirchmann et al. (1991) observed no effect on CO_2 evolution at a nonylphenol concentration of 10 $mg \cdot kg^{-1}$. At 500 $mg \cdot kg^{-1}$ Kirchmann et al. (1991) did see an effect, but in contrast with the results of Trocmé et al. (1988), the effect was an increase in CO_2 evolution by 26%. Kirchmann et al. (1991) also examined effects of nonylphenol on the nitrogen-cycling of microorganisms. No effects on nitrification were observed at a nonylphenol concentration of

10 $mg \cdot kg^{-1}$. With 500 $mg \cdot kg^{-1}$, no effect on nitrogen mineralization occurred, but a 35% decrease in nitrification was observed.

Plants

Toxicity studies have been conducted with a wide range of plants, or plant cell cultures, grown in culture medium (see Appendix IVc). These studies cannot be used in the derivation of a soil quality guideline, and therefore will not be discussed. Nonylphenol toxicity tests using soil have been conducted for five crop species. From a 14 day test with the turnip, *Brassica rapa*, an EC_5 for growth reduction of 10 $mg \cdot kg^{-1}$ was calculated (Günther and Pestemer 1992). Other reported endpoints are one to two orders of magnitude higher. In 21 day tests with nonylphenol, soybean (*Glycine max*), sunflower (*Helianthrus rodeo*), and sorghum (*Sorghum bicolor*) all showed no growth effects at 100 $mg \cdot kg^{-1}$, but had EC_{50}s for growth reduction at 1000 $mg \cdot kg^{-1}$ (Windeatt and Tapp 1986). EC_{50}s for growth reduction have also been reported for lettuce (*Lactuca sativa*) at 559 $mg \cdot kg^{-1}$ after seven days of exposure, and at 625 $mg \cdot kg^{-1}$ after 14 days of exposure (Hulzebos et al. 1993).

Invertebrates

Toxicity tests with nonylphenol have been conducted for three soil-dwelling invertebrates. From a 28 day exposure of the springtail *Folsomia candida*, Madsen et al. (1998) reported that the EC_{50} for reproduction falls between 16 and 30 $mg \cdot kg^{-1}$, and the median lethal concentration is greater than 30 $mg \cdot kg^{-1}$. Researchers have also studied survival and reproduction in another species of springtail, *Folsomia fimetaria*. In 21 day exposures with this second species, Krogh et al. (1996) reported an LC_{10} of 75.1 $mg \cdot kg^{-1}$ and a LC_{50} of 151.3 $mg \cdot kg^{-1}$. For reproduction in *F. fimetaria*, Krogh et al. (1996) reported a 21 day EC_{10} of 23.6 $mg \cdot kg^{-1}$, while Holm (as cited in Jensen et al. 1997) reported EC_{10}s of 27 $mg \cdot kg^{-1}$ and 48 $mg \cdot kg^{-1}$. The EC_{50}s for reproduction in *F. fimetaria* were reported at 65.5 $mg \cdot kg^{-1}$ (Krogh et al. 1996) and 39 $mg \cdot kg^{-1}$ and 59 $mg \cdot kg^{-1}$ (Holm, as cited in Jensen et al. 1997). The effects of nonylphenol on the earthworm *Apporectodea calignosa* in a 21 day exposure was also studied (Krogh et al. 1996). The median lethal concentration occurred at greater than 40 $mg \cdot kg^{-1}$. The median effective concentration for growth reduction was 23.9 $mg \cdot kg^{-1}$. The authors calculated the EC_{10} and EC_{50} for effects on reproduction as 3.44 $mg \cdot kg^{-1}$ and 13.7 $mg \cdot kg^{-1}$, respectively.

Mammals

The only mammals which have been tested for nonylphenol toxicity through ingestion are laboratory rats. In a multigenerational study, Chapin et al. (1999) examined the effects of nonylphenol administered through dosed food on Sprague Dawley rats (*Rattus norvegicus*). At a diet concentration of 650 $mg \cdot kg^{-1}$ (i.e., a dose of 30-108 $mg \cdot kg^{-1}$ body weight) vaginal opening at an earlier age was observed in the F1 generation. Significant effects observed at a diet concentration of 2000 $mg \cdot kg^{-1}$ (i.e., a dose of 100-360 $mg \cdot kg^{-1}$ body weight) included increased relative kidney weights and decreased adult ovary weights in the F1 generation, and increased estrous cycle length in both the F1 and F2 generations. Laws et al. (2000) reported LOECs for increased uterine weight and earlier vaginal opening both at a diet concentration of 50 $mg \cdot kg^{-1}$, and disruption of estrous cyclicity at a diet concentrations of 100 $mg \cdot kg^{-1}$. A LOEC of 75 $mg \cdot kg^{-1}$ body weight per day for increased uterine weight in ovariectomized female rats has been reported by Odum et al. (1999).

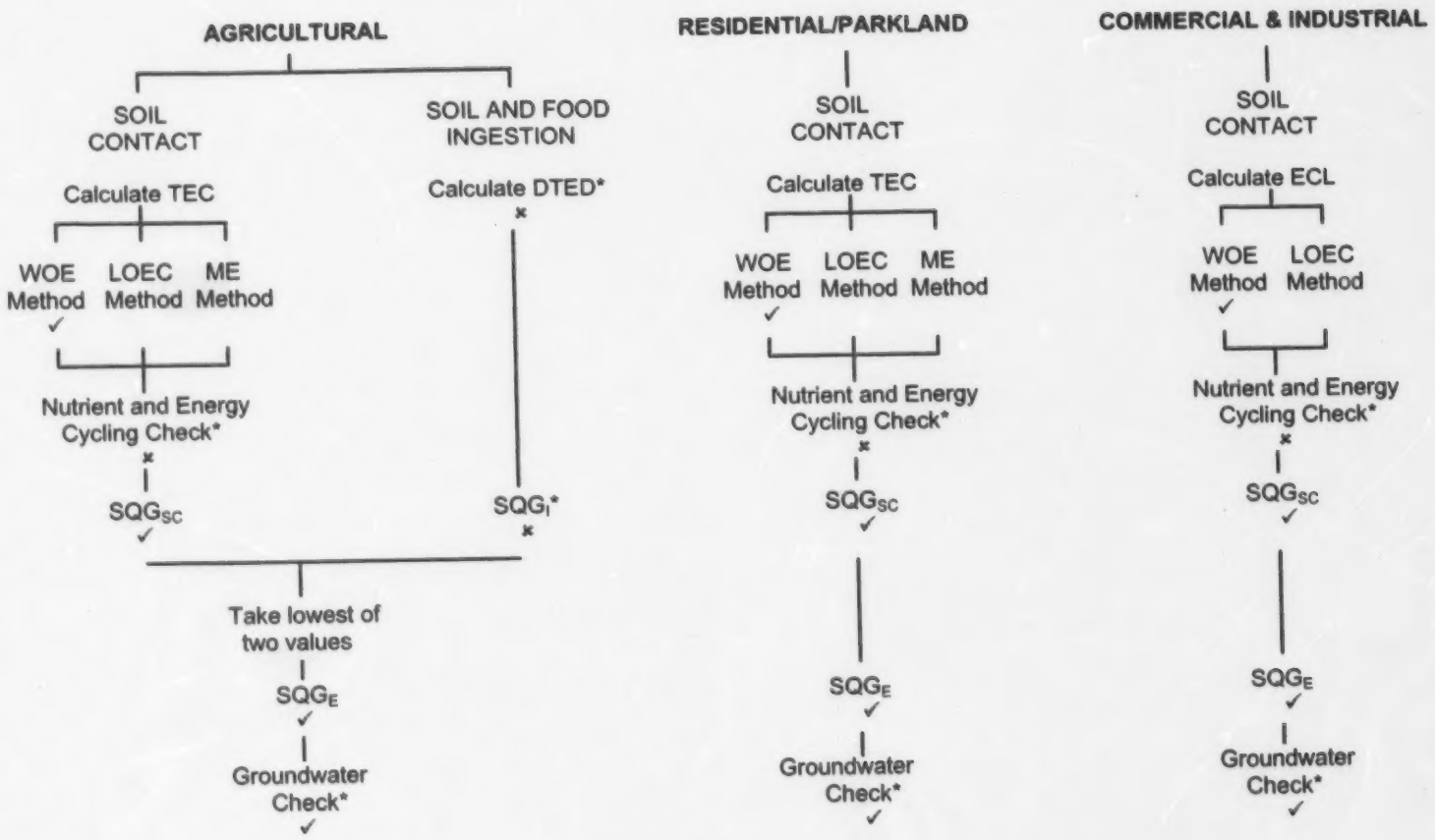


Figure 7. Overview of steps leading to the development of final Environmental Soil Quality Guidelines for nonylphenol.

TEC = threshold effects concentration; DTED = daily threshold effects dose; ECL = effects concentration low;
WOE = weight of evidence; LOEC = lowest observed effect concentration; ME = median effects;
SQG_{SC} = soil quality guideline for soil contact; SQG_I = soil quality guideline for soil and food ingestion;
SQG_E = final environmental soil quality guideline
✓ Indicates steps used in the derivation of guidelines for nonylphenol.
✖ Indicates values that were not calculated in the derivation of guidelines for nonylphenol due to insufficient data.
* Indicates steps that are not mandatory for deriving a guideline if insufficient data is available.

Table 11. Primary data available for toxicity of nonylphenol though direct soil contact.

Species (Common name)	Duration	Endpoint	Effect	Concentration ($mg \cdot kg^{-1}$)	Reference
Plants					
Brassica rapa (turnip)	14d	EC_5	growth	10	Günther and Pestemer 1992
Glycine max (soybean)	21d	NOEC	growth	100	Windeatt and Tapp 1986
	21d	EC_{50}	growth	1000	Windeatt and Tapp 1986
Helianthrus rodeo (sunflower)	21d	NOEC	growth	100	Windeatt and Tapp 1986
	21d	EC_{50}	growth	1000	Windeatt and Tapp 1986
Lactuca sativa (lettuce)	7d	EC_{50}	growth	559	Hulzebos et al. 1993
	14d	EC_{50}	growth	625	Hulzebos et al. 1993
Sorghum bicolor (sorghum)	21d	NOEC	growth	100	Windeatt and Tapp 1986
	21d	EC_{50}	growth	1000	Windeatt and Tapp 1986
Invertebrates					
Apporectodea calignosa (earthworm)	21d	EC_{10}	reproduction	3.44	Krogh et al. 1996
	21d	EC_{50}	reproduction	13.7	Krogh et al. 1996
	21d	EC_{50}	growth	23.9	Krogh et al. 1996
	21d	EC_{10}	mortality	>40	Krogh et al. 1996
	21d	EC_{50}	mortality	>40	Krogh et al. 1996
Folsomia candida (springtail)	28d	EC_{50}	reproduction	16-30	Madsen et al. 1998
	28d	LC_{50}	survival	>30	Madsen et al. 1998
Folsomia fimetaria (springtail)	21d	LC_{10}	survival	75.1	Krogh et al. 1996
	21d	LC_{50}	survival	151.3	Krogh et al. 1996
	21d	EC_{10}	reproduction	23.6	Krogh et al. 1996
	21d	EC_{10}	reproduction	27	Holm*
	21d	EC_{10}	reproduction	48	Holm*
	21d	EC_{50}	reproduction	65.5	Krogh et al. 1996
	21d	EC_{50}	reproduction	39	Holm*
	21d	EC_{50}	reproduction	59	Holm*

* As cited in Jensen et al. 1997

NOEC = no observed effect concentration
EC_x = concentration at which effects are observed in x% of the population
LC_x = concentration that is lethal to x% of the population

Agricultural and Residential/Parkland Land Uses

Soil Contact

There are slight differences in the derivation procedures for soil contact guidelines (SQG_{SC}) among land uses such that a higher level of protection is provided for agricultural and residential/parkland soils than for commercial and industrial soils. For agricultural and residential/parkland land use, derivation of the threshold effect concentration (TEC) due to direct soil contact may proceed by one of three possible methods (weight of evidence method, lowest observed effect concentration method, median effects method), depending on the toxicity data available. A nutrient and energy cycling check is performed (when sufficient data are available) and compared with the TEC. The lower of these two values is adopted as the soil quality guideline for soil contact.

The preferred approach for derivation of the soil contact guideline is to use a weight of evidence method that considers a range of acceptable endpoints. The minimum requirements for this approach are 10 data points from at least three studies, with at least two soil invertebrate species and at least two plant species represented. According to Section 7.5.2.2 of the protocol (CCME 1996), the geometric mean should be used when multiple data are available for the same endpoint with the same species. For the nonylphenol data, geometric means have been applied to the three EC_{10} values and the three EC_{50} values reported for reproduction in springtails (Krogh et al. 1996; Holm as cited in Jensen et al. 1997). The soil toxicity data for nonylphenol satisfied the minimum data requirements with a total of 20 data points (after geometric means were taken for multiple data) from 6 studies representing 5 plant species and 3 soil invertebrate species (see Table 11).

For agricultural and residential/parkland land uses, if the minimum data requirements are met, then the TEC may be calculated by taking the 25th percentile of the frequency distribution (i.e., the "no potential effects" range) and dividing it by an uncertainty factor (if deemed necessary).

Therefore,

$$TEC = NPER \div UF$$

where,
TEC = threshold effects concentration ($mg \cdot kg^{-1}$ soil)
NPER = no potential effects range (25th percentile of distribution)
UF = uncertainty factor (if needed)

Using rank percentile and linear reqression methods, the 25th percentile of the soil contact toxicity data was calculated as 17.15 $mg \cdot kg^{-1}$ soil (Figure 8).

Although there were ample data available representing a wide range of taxonomic groups, the use of an uncertainty factor was deemed necessary. There was a preponderance of median lethal and median effective concentration data that may have introduced some bias into the dataset. Also, more than 25% of the data below the 25th percentile were definitive effects data (i.e., LC_x and EC_x data, as opposed to LOECs and NOECs). Therefore, as recommended in the protocol (CCME 1996), an uncertainty factor of 3 was chosen.

Thus,
$TEC = 17.15 \div 3 = 5.72\ mg \cdot kg^{-1}$ soil $\approx 5.7\ mg \cdot kg^{-1}$ soil

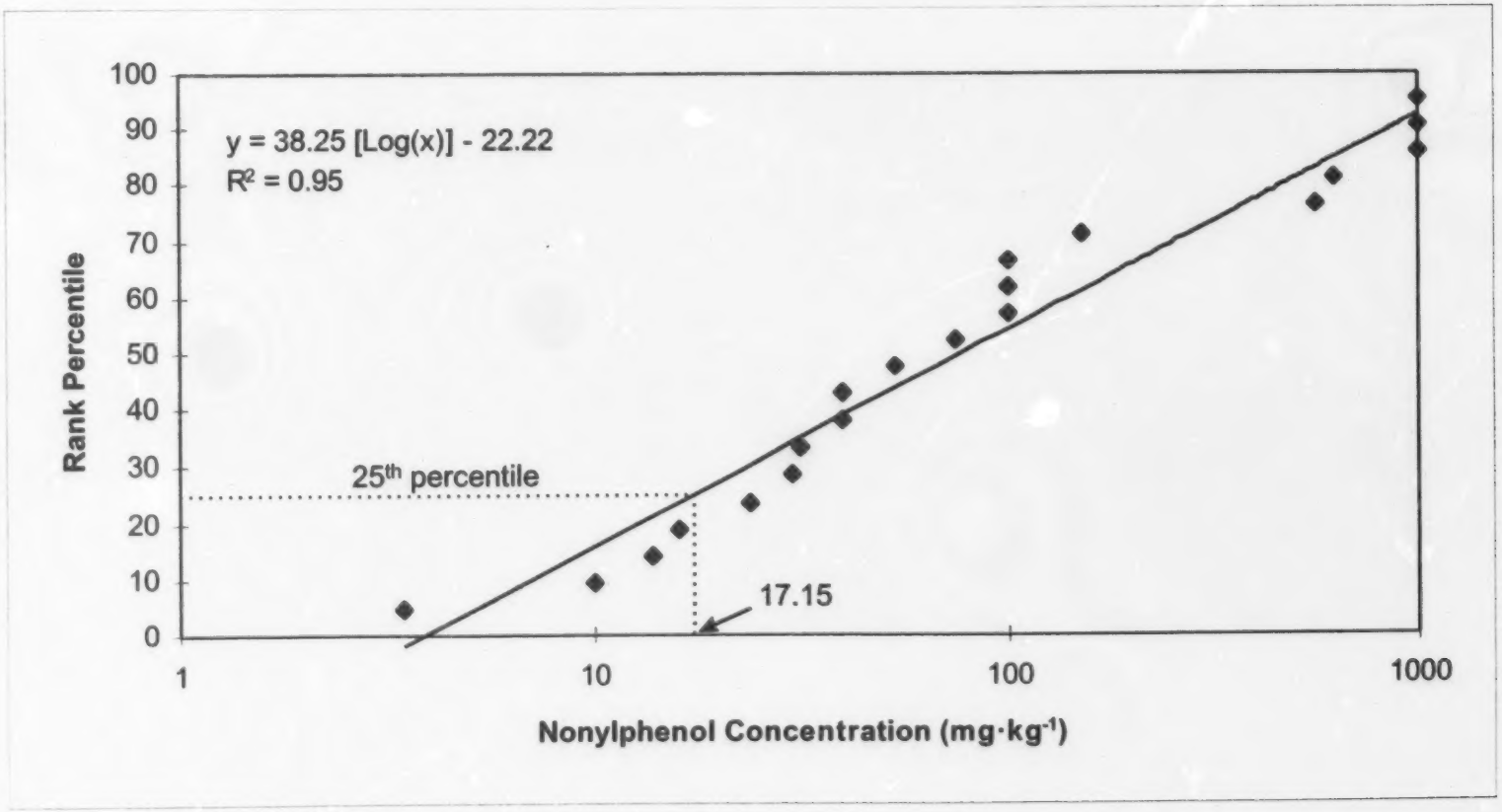


Figure 8. Distribution of soil contact toxicity data used in the guideline derivation for agricultural and residential/parkland land uses. The no potential effects range (i.e., the 25th percentile of the distribution) is indicated by the dotted line.

Nutrient and Energy Cycling Check

Once a soil contact TEC has been calculated, it is then checked against a microbial (nutrient and energy cycling) value. Microorganisms are an essential component of healthy, functioning soils. The ability of soil to support plant life depends on the coordinated activities of microorganisms that mediate processes such as decomposition, respiration, and organic nutrient cycles. The rates of these processes may be affected by contaminants, therefore, the microbial check provides an additional tool for assessing the toxicological impact of soil-associated contaminants on terrestrial life.

The microbial check may be calculated by weight of evidence, LOEC, or median effects methods, depending on available data. Preferred endpoints are nitrogen fixation and nitrification, although carbon cycling and nitrogen mineralization data may also be considered.

The data that were available for microbial responses to nonylphenol are presented in Appendix IVa. Acceptable endpoints included a NOEC and a LOEC for nitrification, a NOEC for nitrogen mineralization, and two NOECs and two LOEC for respiration (Trocmé et al. 1988; Kirchmann et al. 1991). These data were insufficient to meet the minimum requirements for the weight of evidence, LOEC, or median effects approaches, so a nutrient and energy cycling check could not be calculated. It appears though, on the basis of the acceptable LOEC values that are available for microbial processes (ranging from 500 to 1000 $mg \cdot kg^{-1}$), that microorganisms are generally less sensitive to nonylphenol than other terrestrial organisms. Hence, it is unlikely that omission of this check will result in a lack of protection for microbial processes. To calculate the nutrient and energy cycling check using the LOEC method, at least one more study reporting a LOEC value would be required. For the weight of evidence approach, several studies reporting median effects data would be required, preferably with endpoints of nitrogen fixation and nitrification.

As a nutrient and energy cycling check could not be calculated, the soil contact TEC of 5.7 $mg \cdot kg^{-1}$ was adopted as the soil quality guideline for soil contact (SQG_{SC}) for both agricultural and residential/parkland land uses.

Soil and Food Ingestion

The soil quality guideline for soil and food ingestion (SQG_I) applies only to agricultural land use. Derivation of the soil and food ingestion guideline first involves calculating the daily threshold effects dose (DTED). A minimum of three studies is required, at least one of which is an oral avian study, and two of which are oral mammalian studies (no more than one laboratory rodent study, and preferably including an ungulate study). The DTED is calculated as the lowest observed adverse effect level (LOAEL) from the most sensitive species divided by an uncertainty factor. The soil quality guideline for ingestion is then calculated such that the ingestion of soil and plants growing on the soil does not result in the most sensitive species receiving more than 75% of the DTED. More detailed information on these calculations is provided in the protocol (CCME 1996).

There is a lack of data on the toxicity of nonylphenol to grazing livestock and wildlife through ingestion. The few studies that have been conducted have all examined effects on laboratory rodents (Appendix IVd).

The minimum data requirements cannot be met for calculating a soil quality guideline for ingestion (SQG_I). Rough calculations based on the data available suggest that an SQG_I likely would not be lower than the SQG_{SC}; however, due to the high uncertainty involved, no conclusions concerning ingestion can be made at this time.

Commercial and Industrial Land Uses

Soil Contact

For commercial and industrial land use, the effects concentration low (ECL) due to direct soil contact may be calculated using either the weight of evidence method or LOEC method, depending on the toxicity data available. A nutrient and energy cycling check is performed (when sufficient data are available) and the resulting microbial value is compared with the ECL.

As with the agricultural and residential/parkland land uses, the weight of evidence approach was used because the minimum data requirements for this method were met. For commercial and industrial land uses, if the minimum data requirements are met, then the ECL may be calculated by taking the 25th percentile of the "effects" data distribution only (rather than the 25th percentile of the entire data distribution, as is done for the NPER). At this percentile, called the effects range low (ERL), it is expected that some effects will be incurred by soil-dependent biota, but not at the level of median lethality in the population(s). Less stringent protection levels are required than for residential/parkland and agricultural land because the primary land use activities of commercial and industrial lands are not directly dependent on the need to sustain a high level of ecological processes. For this same reason, it is not desirable to achieve lower estimates of the ECL using uncertainty factors.

Therefore, the effects concentration low (ECL) for nonylphenol was calculated as

$$ECL = ERL$$

where,
ECL = effects concentration low (mg·kg^{-1} soil)
ERL = effects range low (25th percentile of effects data distribution, in mg·kg^{-1} soil)

Using ranked percentile and linear regression methods, the 25th percentile of the soil contact effects data was calculated as 14.16 mg·kg^{-1} soil (Figure 9).

Thus,
ECL = 14.16 mg·kg^{-1} soil ≈ 14 mg·kg^{-1} soil

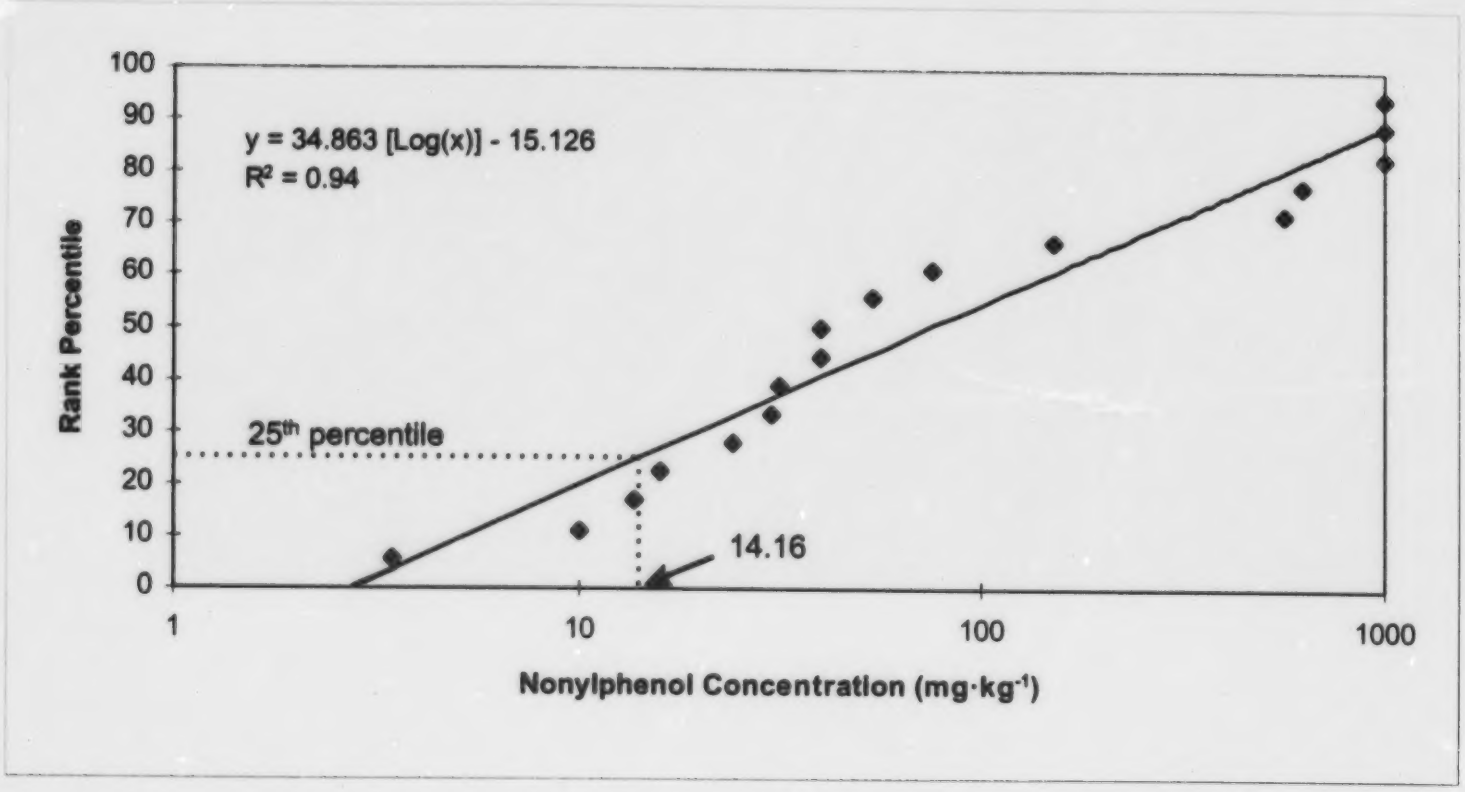


Figure 9. Distribution of soil contact effects toxicity data used in guideline derivation for commercial and industrial land uses. The effects range low (i.e., the 25th percentile of the effects data distribution) is indicated by the dotted line.

Nutrient and Energy Cycling Check

Once a soil contact ECL has been calculated, it is then checked against a microbial (nutrient and energy cycling) value. The microbial check may also be calculated by either the weight of evidence method or the LOEC method, depending on available data. Preferred endpoints are nitrogen fixation and nitrification, although carbon cycling and nitrogen mineralization data may also be considered. If the microbial value is lower than the ECL, then significant adverse effects may be experienced by microorganisms controlling nutrient and energy cycling processes. In this case, the geometric mean of the two values is taken and used as the soil quality guidelines for soil contact (SQG_{SC}) for commercial and industrial land use. However, if the microbial value is above the ECL, then a level of effects consistent with the objective of this procedure is expected and the ECL is adopted directly as the SQG_{SC} for commercial and industrial land use.

The data that were available for microbial responses to nonylphenol are presented in Appendix IVa. These data were insufficient to meet the minimum requirements for either the weight of evidence method or the LOEC method. To calculate the nutrient and energy cycling check using the LOEC method, at least one more study reporting a LOEC value would be required. For the weight of evidence approach, several studies reporting median effects data would be required, preferably with endpoints of nitrogen fixation and nitrification.

As a nutrient and energy cycling check could not be calculated, the soil contact ECL of 14 $mg \cdot kg^{-1}$ was adopted as the soil quality guideline for soil contact (SQG_{SC}) for both commercial and industrial land uses.

Groundwater Check

A major concern with soil contamination is that it can lead to contamination of groundwater. Soil quality guidelines for all four classes of land use must be designed to account for possible transfers to groundwater such that concentrations of a substance in groundwater do not exceed the water quality guidelines for the protection of freshwater aquatic life. To prevent such an exceedence, a groundwater check is calculated based on equilibrium partitioning between solid and aqueous phases. The applicability of equilibrium partitioning to nonylphenol is discussed in Chapter 6. For non-ionic organics, such as nonylphenol, the following equation (CCME 1996) is used:

$$Y = DF \cdot C_{wa} \cdot (K_d + \theta_m)$$

where,

Y = concentration of nonylphenol in soil (mg NP·kg^{-1} dry soil)
DF = dilution factor = 50 (default)
C_{wa} = water protection value = Canadian water quality guideline ($mg \cdot L^{-1}$)
K_d = distribution coefficient
θ_m = soil moisture content = 0.1 (default)

The distribution coefficient (K_d) is directly related to the organic matter content and can be derived using the sorption partition coefficient (K_{oc}) and the organic fraction of dry soil (f_{oc}) as described by the equation:

$$K_d = K_{oc} \cdot f_{oc}$$

A default value of 0.3% (or 0.003) is used for f_{oc} (CCME 1996). The log K_{oc} for nonylphenol has been estimated to range from 4.7 to 5.6 (Sekela et al. 1999), so, a mean K_{oc} can be determined by taking the anti-log of these two values and then calculating the geometric mean.

Therefore,

K_{oc} = 141 254 $L \cdot kg_{oc}^{-1}$
K_d = 423.8 $L \cdot kg_{soil}^{-1}$

For the protection of freshwater aquatic life, a water quality guideline of 1.0 $\mu g \cdot L^{-1}$ (or 0.001 $mg \cdot L^{-1}$) was developed for nonylphenol in Chapter 5. Therefore:

$$Y = (50)(0.001)(423.8 + 0.1)$$
$$= 21.2 \text{ mg NP} \cdot kg^{-1} \text{ dry soil}$$

The groundwater check indicates that for the protection of freshwater aquatic life, the level of nonylphenol in soil should not exceed 21 $mg \cdot kg^{-1}$.

Off-site Migration Check

Contaminated soil can be transferred from one property to another through erosion by water and wind with subsequent deposition. This transfer of contaminants can result in unacceptable degradation of an adjacent property that was previously uncontaminated, or used for a more sensitive land use. Therefore, an off-site migration check was calculated to determine the concentration of nonylphenol in soil from an industrial site that could, through erosion, hypothetically result in levels on an adjacent residential/parkland property that exceed the residential/parkland SQG_E. The check value was calculated using the following equation (CCME 1996):

$$C_i = (14.3 \cdot SQG_{RP}) - (13.3 \cdot BSC)$$

where,
C_i = concentration of nonylphenol in the eroded soil (mg NP$\cdot kg^{-1}$)
SQG_{RP} = soil quality guideline for residential/parkland land use
BSC = background concentration of nonylphenol in the receiving soil (mg NP$\cdot kg^{-1}$)

The soil quality guideline for soil contact for residential/parkland land uses is 5.7 $mg \cdot kg^{-1}$, and the background concentration of nonylphenol is assumed to be zero. Substituting these values into the equation yields the following off-site migration check value:

$$C_i = 82 \text{ mg NP} \cdot kg^{-1}$$

The off-site migration check indicates that for the protection of adjacent properties, the level of nonylphenol in soil on industrial sites should not exceed 82 $mg \cdot kg^{-1}$.

Environmental Soil Quality Guideline Derivation (SQG_E)

The final environmental soil quality guideline for agricultural land use is determined by taking the lower of either the soil contact guideline (SQG_{SC}) or the soil ingestion guideline (SQG_I), if both are available. If there are not enough data to calculate a soil ingestion guideline, then the soil contact guideline is taken as the final environmental guideline; however, the final environmental guideline cannot be derived if there is insufficient data for the soil contact guideline. For all other soil uses, the soil contact guideline is used as the final environmental soil quality guideline.

Because no data were available on toxicity of nonylphenol through ingestion of food or soil, the SQG_{SC} of 5.7 $mg \cdot kg^{-1}$ will be taken as the final SQG_E for agricultural land use. For the other three land uses, the SQG_{SC} is used as the SQG_E also. Therefore, the SQG_Es for residential/parkland, commercial, and industrial land uses are 5.7 $mg \cdot kg^{-1}$ soil, 14 $mg \cdot kg^{-1}$ soil, and 14 $mg \cdot kg^{-1}$ soil, respectively. The groundwater check value was higher than all of the SQG_E values, indicating that these guidelines are sufficiently low to protect against contamination of groundwater. The off-site migration check value was higher than the SQG_E for industrial land uses, indicating that this

guideline is sufficiently low to protect against degradation of adjacent lands through deposition of eroded soil.

Recommended Canadian Soil Quality Guidelines

The final Canadian Soil Quality Guidelines for a substance are determined as the lower of the environmental soil quality guideline (SQG_E) and the human health soil quality guideline (SQG_{HH}) for each land use. Because human health soil quality guidelines have not been developed for NP and NPEs, the environmental guidelines will be taken as the final Canadian Soil Quality Guidelines for nonylphenol and its ethoxylates. A summary of the soil quality guideline values for each land use type is provided in Table 12. These guidelines are expressed in nonylphenol toxic equivalency units (NP TEQs) and may be applied to mixtures of nonylphenol and nonylphenol ethoxylates. The total concentration of nonylphenolic substances in a soil sample (in NP TEQs) should be calculated using the nonylphenol toxic equivalency factors provided in Chapter 3 (Table 4). Further guidance and recommendations for the implementation of these guidelines are provided in Chapter 9.

The Canadian environmental soil quality guidelines recommended for agricultural and residential/parkland land use are higher than the Danish soil quality criterion for nonylphenol (see Table 8). The Danish criterion was based on the 21 day EC_{50} for reduced reproduction in earthworms at 13.7 $mg \cdot kg^{-1}$ as determined by Krogh et al. (1996). This endpoint, which represented the lowest toxicity value in their data set, was divided by an application factor of 1000 and rounded off to 0.01 $mg \cdot kg^{-1}$. It is due to this extremely conservative application factor that the Danish soil criterion is so much lower than the Canadian soil guideline. Both Sweden and Denmark have also set limits for NP in sludge used as agricultural fertilizer (Table 8). The Danish guidelines refer to the total concentration of 4-NP, NP1EO and NP2EO in sludge (European Commission 1999; Danish Ministerial Order No. 823, Sept. 16, 1996). The higher guideline values for sludge, compared with soil, reflect the fact that the nonylphenol concentrations in the sludge will be effectively diluted through the application of the sludge over a large area of soil.

Table 12. Summary of soil quality guideline derivation for nonylphenol. *

Guideline ($mg \cdot kg^{-1}$)	Land use			
	Agricultural	Residential/ Parkland	Commercial	Industrial
Final Canadian SQG	5.7	5.7	14	14
SQG_E	5.7	5.7	14	14
SQG_{SC}	5.7	5.7	14	14
Nutrient and energy cycling	NC	NC	NC	NC
SQG_I	NC	NA	NA	NA
Groundwater check (freshwater aquatic life)	21	21	21	21
Off-site migration check	NA	NA	NA	82
SQG_{HH}	NC	NC	NC	NC

* All guideline values are expressed in toxic equivalent (TEQ) units.
SQG_E = soil quality guideline for environmental health; SQG_{SC} = soil quality guideline for soil contact; SQG_I = soil quality guideline for soil and food ingestion; SQG_{HH} = soil quality guideline for human health
NC = not calculated; NA = not applicable

CHAPTER 8. AREAS FOR FUTURE RESEARCH

The guidelines developed in this document are based on the best available data. In the process of deriving these guidelines, however, it was noted that there are certain areas of study in which limited data are available for nonylphenol and its ethoxylates. This chapter highlights some of the gaps in our knowledge of these substances with the hope that it will provide stimulation and direction for further research.

Concentrations in Canada

Historically, concentrations of NP and NPEs have not been routinely monitored in the Canadian environment. Consequently, there is limited data concerning the distribution and levels of these substances that occur in Canada. In particular, there is either no data, or very limited data, that exist for Canadian marine and estuarine waters, marine sediments, groundwaters, soils, and industrial effluents and sludges. There is also a lack of data on levels of NPECs and NP*n*EOs ($n \geq 3$) in freshwater sediments.

Fate and Behaviour

There is a need for further studies into the fate and behaviour of NP and NPEs in the environment. In particular, more field studies on the fate of these substances in sediments are needed because most work to date has been conducted in a laboratory setting. There does not appear to have been any work done on the effects of photolysis on NP/NPEs present on soil surfaces. There also has been no research into the effects of varying pH on the aquatic toxicity or bioavailability of nonylphenol. Further investigation into the biological fate of NP and NPEs is also needed. Specifically, the data available on uptake and elimination rates in invertebrates, uptake by aquatic plants, and uptake by terrestrial animals are limited.

Toxicity Testing

An area of research that has been largely unexplored is the toxic effects of NP and NPEs on mammalian and avian consumers of aquatic life. There are very limited toxicity data available for sediment-dwelling organisms. Toxicity testing has also not been conducted for terrestrial wildlife and grazing livestock.

The toxicity data that does exist for aquatic algae, plants, invertebrates and vertebrates is largely for NP, NP1-2EO, NP9-10EO, and, to a lesser degree, NP1-2EC. There is a need for more toxicity data on other NPE oligomers, particularly those containing between 3 and 8 ethoxylate groups.

Further investigation into the mode of toxicity for these substances would also be useful. At present, the exact mechanisms of toxicity via narcosis are unclear. There is also some controversy over the exact mechanisms involved in the endocrine disrupting effects produced by these substances.

Guideline Derivation and Implementation

When more toxicity data becomes available, it may be possible to upgrade the status of the marine interim WQG developed in this document. A full marine WQG could be derived if more chronic toxicity data becomes available for marine fish and for marine invertebrates other than crustaceans.

An unconventional approach (equilibrium partitioning) was used to derive the sediment quality guidelines in this document due to a lack of sediment toxicity data. In the future, if more sediment toxicity data becomes available for nonylphenol it may be possible to derive guidelines which are based on approaches in the CCME protocol (CCME 1995). In order to use the NSTP approach,

12 more entries in the effect data set and 3 more entries in the no-effect data set would be required for the freshwater SQG; 11 more entries in the effect data set and 6 more entries in the no-effect data set would be required for the marine SQG. Alternatively, the freshwater SQG could be derived using the SSTT approach if three more studies on sediment-resident invertebrates are conducted, including at least one study on a benthic amphipod, and at least one partial or full life-cycle test that reports ecologically relevant sub-lethal endpoints. A marine SQG could be derived using the SSTT approach if at least two more studies on sediment-resident invertebrates are conducted, at least one of which is a partial or full life-cycle test with sub-lethal endpoints.

The soil quality guidelines in this document were developed without the calculation of either a soil and food ingestion guideline, or a nutrient and energy cycling check. The soil and food ingestion guideline could be calculated in future if at least one oral avian study was conducted, and at least one oral mammalian study was conducted with a mammal other than a laboratory rodent, but preferably with an ungulate species. To calculate a nutrient and energy cycling check, at least one more study reporting a LOEC value would be required, or several studies reporting median effects data for endpoints such as nitrogen fixation and nitrification.

This document recommends the use of TEF values that were developed by Servos et al. (2000). Further work is needed to verify and refine these TEF values. For example, a single TEF is currently given for the entire group of NPEs ranging from NP9EO and up. It may be possible to develop oligomer-specific TEFs if variations in toxicity are known to occur for different ethoxylate chain lengths. Due to a lack of toxicity data for NPEs with 3 to 8 EO groups, a conservative TEF value was estimated from the other TEFs. Further work is need to develop TEFs for this group of ethoxamers.

At present, we are recommending that OP, OPEs, and OPECs be included in the TEQ approach for nonylphenolic substances. Further investigation is needed to confirm whether these substances should be grouped together, or whether they warrant separate guidelines. As with NPEs, further refinement and validation of the TEF values for OPEs is needed.

CHAPTER 9. GUIDANCE ON IMPLEMENTATION

General Guidance for all Guidelines

The legislative authority for implementation of Canadian Environmental Quality Guidelines (EQGs) lies with each provincial and territorial jurisdiction (with the exception of federal lands). The provincial and territorial governments may develop their own science-based criteria, guidelines, objectives and standards, but often the Canadian EQGs form the scientific basis for these (CCME 1999). Canadian EQGs may also form the basis for federal use-specific guidelines and objectives.

The Canadian EQGs developed in this document for nonylphenol should be used along with all other relevant information in making practical and informed decisions regarding water, sediment, and soil quality. It is widely recognized that no single assessment tool can always be used to predict whether adverse biological effects will occur as a result of contaminant exposure. Rather, the appropriate use of several different tools provides the most useful information (Chapman 1995). Apart from EQGs, other scientific tools available to assist in the protection and management of water, sediment, or soil quality include other assessment values (such as the PEL for sediment), biological assessments, or guidelines for other media.

It should also be noted that socio-economic or technological factors that may influence the application of Canadian EQGs are not considered in their development process. However, such factors may play a varying role in the application of EQGs (and/or in the development of site-specific objectives) within the decision-making framework of different jurisdictions and programs (Gaudet et al. 1995).

Negligible risk to biota, their functions, or any interactions that are integral to sustaining the health of ecosystems and the designated resource uses they support, should result when nonylphenol (and other nonylphenolic substances) are present in the ambient environment at the concentrations recommended by the Canadian EQGs developed in this document. It should be noted, however, that for ecosystems of superior quality, impairment to these guideline concentrations is not advocated.

Additional general information on the use of Canadian EQGs is available in the introductory chapter of the publication *Canadian Environmental Quality Guidelines* (CCME 1999). More specific guidance, relevant to the guidelines for nonylphenol and its ethoxylates, is provided below.

Application of the TEQ Approach to EQGs

When comparing environmental samples to the guidelines developed in this document, it is important that concentrations of NP and all NPEs and NPECs in the sample are measured, converted to nonylphenol toxic equivalents (NP TEQs), and then summed. It is also recommended that OP, OPEs and OPECs, if present in the sample, be included in the calculation of the total NP TEQ. Toxic equivalency factors (TEFs) are provided in Table 4 of Chapter 3. The following example illustrates the proper use of the TEQ approach and its application towards the Canadian Environmental Quality Guidelines for nonylphenol and its ethoxylates.

Consider a water sample collected from a freshwater stream that has been analysed for all possible nonylphenolic substances. The sample was found to contain 0.1 $\mu g \cdot L^{-1}$ of NP, 1.0 $\mu g \cdot L^{-1}$ of NP1EO, 0.2 $\mu g \cdot L^{-1}$ of NP3EO, and 2.0 $\mu g \cdot L^{-1}$ of NP1EC. The TEF values for toxicity of NP, NP1EO and NP1EC are 1, 0.5, and 0.005, respectively, and we will assume a relative potency of 0.5 for NP3EO (see Table 4). Therefore, using the equation

Total concentration = Σ (C_x x TEF),

and plugging in the concentrations from our water sample, we get:

Total concentration = (0.1 $\mu g \cdot L^{-1}$ x 1) + (1.0 $\mu g \cdot L^{-1}$ x 0.5) + (0.2 $\mu g \cdot L^{-1}$ x 0.5) + (2.0 $\mu g \cdot L^{-1}$ x 0.005)
= 0.71 $\mu g \cdot L^{-1}$

Therefore, our water sample contains 0.71 $\mu g \cdot L^{-1}$ of nonylphenolic substances, as expressed in NP TEQ units. This total concentration 0.71 $\mu g \cdot L^{-1}$ is the value that should then be compared against the nonylphenol Canadian Water Quality Guideline for the protection of freshwater aquatic life (i.e., 1.0 $\mu g \cdot L^{-1}$). In this example then, the water sample does not exceed the freshwater guideline for nonylphenol and its ethoxylates and adverse effects to aquatic life are not expected to occur in this water.

These same NP TEF values should be used to determine total NP TEQs for sediment and soil samples before comparing them to the respective guideline values. Narcosis is a common mode of toxicity for nonylphenolic substances across all taxonomic groups (see earlier discussion under "Modes of Toxicity" in Chapter 3), so the NP TEF values should be applicable to plants, invertebrates, fish, mammals, etc., regardless of whether they are water-, sediment-, or soil-dwelling species.

Water Quality Guidelines

The toxicological data upon which these guidelines are based were conducted within the pH ranges of 5.6 to 8.9 and 7.3 to 9.6 for fresh and marine waters, respectively. Most natural waters will have pH values within these ranges. However, site-specific considerations may be needed for those locations where the water pH falls outside of these ranges. In particular, these guidelines may not be sufficiently protective for highly alkaline waters. The acid dissociation constant (pK_a) of nonylphenol is 10.7, indicating that in most natural waters NP will remain predominantly in the undissociated form. In waters with a pH greater than 10.7, the dissociated form, which is more soluble and may differ in bioavailability or toxicity, will be present. It is currently unknown whether pH has any effect on the aquatic toxicity or bioavailability of NP and NPEs.

The presence or absence of sensitive species at a local site is another factor that might play a role in the development of site-specific objectives.

These guidelines refer to concentrations in unfiltered samples.

Sediment Quality Guidelines

The interim sediment quality guidelines presented here represent the concentrations of nonylphenol or nonylphenol equivalents in sediments below which adverse effects in sediment-dwelling aquatic biota are not expected to occur. Although these interim sediment quality guidelines are considered provisional at this time, they should not be used differently than they would be if they were full sediment quality guidelines. The revision and re-assessment of these provisional ISQGs will be possible when sufficient data become available to support the use of the modified NSTP or SSTT approaches as described in the formal protocol (CCME 1995).

Using the equilibrium partitioning approach, the provisional interim sediment quality guidelines were normalized to 1% TOC (f_{oc} = 0.01) in order to express them in terms of sediment dry weight. In jurisdictions where the percentage of organic carbon in sediments is known to be consistently higher or lower than 1%, local estimates of TOC may be used to set site-specific sediment objectives. This is done by multiplying the ISQG by the percentage of organic carbon. For example, for freshwater sediments with 2% TOC, the freshwater ISQG of 1.4 $mg \cdot kg^{-1}$ would be multiplied by 2 to obtain a site-specific objective of 2.8 $mg \cdot kg^{-1}$. With lower levels of organic carbon there will be less binding of contaminants to the sediments, and consequently greater bioavailability. Therefore, it is important that the f_{oc} value used is based on the lowest sediment organic carbon concentration that might possibly occur, such that all aquatic life in the designated

jurisdiction will be protected. It is not recommended that f_{oc} values outside of the range 0.002 to 0.12 be used in deriving site-specific objectives. For sediments that contain less than 0.2% or greater than 12% TOC, other factors that the equilibrium partitioning method does not account for may influence the contaminant partitioning (USEPA SAB 1992). Further generic guidance regarding the implementation of Canadian sediment quality guidelines is provided in CCME (1995).

Soil Quality Guidelines

The toxicological studies upon which these guidelines are based did not report soil pH in many cases; however, where reported, the studies were conducted within the pH range of 5.8 to 7.5. Therefore, site-specific consideration may be required for soils that fall outside of this pH range.

In particular, these guidelines may not be sufficiently protective for sites with highly alkaline soils (i.e., with a pH greater than 10). The high acid dissociation constant of nonylphenol (pK_a = 10.7) indicates that in most natural environments NP will remain predominantly in the undissociated form. Extremely high pHs may create dissociated forms that are less readily sorbed than the parent nonylphenol, and much more mobile in soil. In these cases, the groundwater check as utilized in this document will not be applicable (see CCME 1996), and the development of more conservative site-specific guidelines than presented here may be necessary to ensure adequate protection.

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ANNEX I. ECOLOGICAL RELEVANCE OF ENDOCRINE DISRUPTION

Environmental guidelines must be based on toxicity data that have some ecological relevance. Conventional toxicity endpoints, such as mortality, growth inhibition, and reduced reproductive output are widely acknowledged as ecologically-relevant effects that can cause impacts at organizational levels higher than the individual (i.e., population, community and ecosystem levels). There has recently been much debate over the ecological relevance of effects caused by endocrine disrupters. Endocrine disrupting chemicals (EDCs) work at various biochemical levels, such as affecting the synthesis of hormones, hindering movement of hormones through the blood by hormone transporting proteins, upsetting the metabolism of hormones, or by directly interfering with cellular hormone receptors (Bjerregaard et al. 1998). However, even though an EDC may cause biological and physiological changes within an organism, whether or not these changes affect whole organism performance in terms of survival, reproduction or growth will depend on the hormone affected, the degree of impairment, the timing of the exposure in terms of the organism's life stage, and the relative sensitivity of the particular species (Van Der Kraak 1998).

Evidence of endocrine disruption, in various forms, has been extensively documented in vertebrates such as fish, marine mammals, predatory birds, and even alligators (Fry and Toone 1981; Reijnders 1986; Brouwer et al. 1989; Munkittrick et al. 1991, 1992; Jobling and Sumpter 1993; Purdom et al. 1994; Fry 1995; Guillette et al. 1994, 1995, 1996; Harries et al. 1997). There has been less documentation of endocrine disruption in invertebrates species; most cases have involved daphnids or marine molluscs (Taylor et al. 1999). Although there is a wealth of data describing the effects of endocrine disrupters at the cellular and biochemical levels, very few studies have investigated the long-term ramifications of these effects at the population or community level (Hutchinson and Matthiessen 1999). In some cases, although population-level changes have been observed, the effects have not been linked to a specific chemical.

Ankley and Giesy (1998) have developed a weight of evidence approach for conclusively demonstrating ecologically relevant effects of an EDC. First, there must be documentation of effects in individuals and of adverse effects in populations. Second, there must be a connection between the effects observed in populations and those observed in individuals. Third, a plausible mode of action must be identified that is consistent with the effects observed in the individuals. Fourth, there must be positive identification of specific contaminants operating through this mode of action. Fifth, the exposure metrics must be reasonable in terms of individual-level effects occurring via the mode of action under consideration. And finally, there should be evidence that the population or individuals will recover upon removal of the chemical stressor.

For NP, short-chain NPEs and NPECs, there is extensive documentation of effects at the individual level, and the mechanism of action is generally understood. However, how these effects on individuals may translate into adverse effects at the population level is not well understood.

In the case of fish, to determine the significance for populations of endocrine disrupter effects such as vitellogenin induction and intersex states, it will first be necessary to establish their impacts on individual fitness (Taylor et al. 1999). It is important to establish possible links between the vitellogenin response and ecologically important parameters that are relevant to population dynamics, such as semen quality, testicular development, histological changes and offspring sex ratio and development (Bjerregaard et al. 1998). A few studies have examined the effects of xenobiotically-induced vitellogenesis on the individual fitness of fish. Carragher and Sumpter (1991) found that when they induced vitellogenin synthesis in juvenile rainbow trout with 17ß-estradiol, there was a significant decrease in the amount of calcium in the fish scales, and a significant increase in plasma calcium levels (possibly for incorporation into vitellogenin). The authors suggested that at higher demands for calcium due to vitellogenesis, the scales could become loosened, thus compromising the fish's defenses. Therefore, there may be a link between vitellogenesis and fish survival rates (Carragher and Sumpter 1991). Herman and

Kincaid (1988) found that when rainbow trout underwent prolonged vitellogenin induction due to ß-estradiol exposure, mortality rates increased, and necrosis and damage to the kidney and liver tissues were severe. Induction of vitellogenin in male fish has also been correlated to decreases in testicular growth and serum testosterone levels (Jobling et al. 1996; Folmar et al. 1996). These effects might have a bearing on reproductive success.

In their multigenerational study with rats, Chapin et al. (1999) observed increased estrous cycle length in those individuals exposed to NP. This estrogenic response could have adverse effects at the population level because lengthened estrous cycles have been associated with reduced fecundity (Chapin et al. 1997).

It seems likely that these estrogenic effects of NP and short-chain NPEs and NPECs could result in population-level effects. However, at this point such effects are largely speculative and actual documentation of population-level effects in the field are needed.

In some cases, population-level effects have been observed due to NP, but further research is needed to confirm that these effects are the result of endocrine disruption. For example, adverse effects of NP on the smoltification of juvenile Atlantic salmon (*Salmo salar*) have been observed in laboratory studies (Madsen et al. 1997; Brown et al. 1998), and resultant decreases in population sizes have been documented in the field (Fairchild et al. 1999). Although the authors suspect endocrine disruption, the mechanism by which NP affects smoltification is unknown.

In an attempt to address the current data gaps concerning the ecological impacts of EDCs, there has been a call for more multigenerational toxicity tests with suspected endocrine disrupters (Taylor et al. 1999). Studies examining population parameters such as offspring survival and recruitment are needed to assess the effects of long-term exposure to EDCs. The OECD is currently working to develop and validate test methods for assessing the potential of new and existing chemicals for endocrine disruption (Taylor et al. 1999).

Endocrine disruption endpoints were considered in the development of the guidelines in this document. In the aquatic toxicity dataset, the range of concentrations at which EDC effects were observed were comparable with the range of concentrations for more conventional endpoints. The lowest primary toxicity data available for both the freshwater and marine Water Quality Guidelines used conventional endpoints, so, there was no need to decide whether a guideline could be based on endpoints related to endocrine disruption, and whether these endpoints are ecologically relevant.

If future research concludes that EDC effects due to NP and short-chain NPEs do result in ecologically relevant impacts on populations, then there may be a need to re-examine the toxic equivalency factors that are recommended in this document. Servos et al. (2000) calculated relative estrogenicity values for various alkylphenolic compounds, relative to NP. These relative estrogenicity values for NP1EO, NP2EO, NP*n*EO ($n \geq 9$), NP1EC, NP2EC, OP, OP1EC and OP2EC are 0.67, 0.67, 0, 0.63, 0.63, 4.1, 0.63 and 0.63, respectively. These relative estrogenicities differ from the TEF values based on conventional toxicity endpoints. For example, although NPECs have a low relative conventional toxicity, with TEFs of 0.005, they are considerably more estrogenic, with relative estrogenicity values estimated at 0.63. Octylphenol, though similar to NP in toxicity, is about four times more estrogenic. Therefore, environmental samples which do not exceed the guidelines based on conventional toxicity TEFs, could still presumably cause adverse effects due to estrogenic effects, particularly if they contain significant quantities of OP, OPECs, or NPECs. In future, this is an issue that will need to be resolved as more and more information on EDCs is coming to light.

ANNEX 2. DATASET REQUIREMENTS FOR DERIVING WATER QUALITY GUIDELINES

Water quality guidelines are based on a single, ecologically-relevant, toxicity endpoint for the most sensitive life stage of the most sensitive aquatic species investigated. To determine the concentration that represents the most sensitive and protective toxicity endpoint, toxicological data are evaluated according to a number of screening criteria. The screening criteria (e.g., acceptable experimental design, laboratory procedures, documentation of relevant experimental conditions and parameters) have been described in detail in the protocol (CCME 1991; CCME 1999). Toxicity studies are classified as primary, secondary, or unacceptable. Only primary studies may be used in the actual derivation of the WQG. Secondary studies, however, may be used to help meet the minimum toxicological data set requirements.

Studies which were identified as being of primary quality, and therefore could be used in guideline derivation, met all of the following criteria:

1. flow-through or renewal test conditions were used (rather than static)
2. the study was conducted under controlled laboratory conditions rather than in the field
3. water parameters, such as temperature, pH, and hardness, were measured and reported
4. sufficient replication was used
5. more than a single concentration of NP was tested
6. concentrations of NP in the water were measured (i.e., did not just report nominal concentrations)
7. conventional endpoints with documented ecological relevance were used
8. dose-dependent concentration responses were observed
9. experimental conditions and/or analytical methods were described sufficiently to evaluate the validity of the study (note: data obtained from published abstracts, citations in other articles/reports, or from articles published in foreign languages were considered unacceptable)

The following discussion summarizes the minimum data set requirements for development of freshwater and marine WQGs.

Freshwater

For a full freshwater life guideline, the toxicological data set of primary studies must minimally include the following:

- at least three studies on three or more freshwater fish species resident in North America, including at least one cold-water species (e.g., trout) and one warm-water species (e.g., fathead minnow), and at least two must be chronic (partial or full life-cycle) studies
- at least two chronic (partial or full lifecycle) studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in North America (e.g., daphnid).
- at least one study of a freshwater vascular plant or freshwater algal species resident in North America (note: for highly phytotoxic variables, four acute and /or chronic studies on nontarget freshwater plant or algal species are required)

The primary data available for freshwater toxicity due to nonylphenol met all of the minimum dataset requirements.

Marine

The minimum requirements for a full marine guideline are the same as those for freshwater life except that the studies must include temperate marine fish, invertebrates and plants/algae. Also, the invertebrate studies must include two species from different classes, but it is not necessary that one of those species be planktonic.

The marine toxicity dataset for nonylphenol was unable to meet these minimum requirements, and therefore a full marine guideline could not be derived.

When the minimum data requirements for derivation of a full guideline cannot be met, it may still be possible to derive an interim guideline. The minimum data set requirements for an interim marine life guideline are:

- at least two acute and/or chronic studies on two or more marine fish species, one of which is a temperate species
- at least two acute and/or chronic studies on two or more marine invertebrate species from different classes, one of which is a temperate species

Secondary studies, as well as primary studies, may be used to meet the minimum requirements for an interim guideline.

Studies which were considered of secondary quality, and therefore could be used to meet the minimum data requirements for an interim guideline, met the same criteria as the primary studies, except for one or more of the following characteristics:

1. test conditions were static (rather than flow-through or renewal)
2. only nominal (rather than measured) concentrations of NP in water were reported
3. ecologically relevant endpoints other than survival, reproduction or growth were reported

There were sufficient marine toxicity data to meet the minimum requirements for the derivation of an interim guideline.

ANNEX 3. SUMMARY OF SEDIMENT QUALITY GUIDELINE PROTOCOL

There are three approaches to deriving sediment quality guidelines that are recommended in the CCME protocol: the National Status and Trends Program Approach, the Spiked-Sediment Toxicity Test Approach, and adoption of guidelines from other jurisdictions (CCME 1995). The data available for nonylphenol did not support the use of any of these three approaches, and therefore they were not used in the final derivation of Canadian Sediment Quality Guidelines for nonylphenol and its ethoxylates. This annex provides a brief summary of the three protocol approaches and includes a discussion of the nonylphenol sediment toxicity data that would be needed to support the use of these guideline derivation methods.

The Modified National Status and Trends Program (NSTP) Approach

The NSTP approach to deriving sediment quality assessment values was originally developed by Long and Morgan (1990). This approach was adopted, with some modifications, for use in developing sediment quality guidelines in Canada (CCME 1995) and is therefore referred to in this document as the *modified* NSTP approach. Modifications to this approach include the separate evaluation of information for freshwater and marine systems, an expansion of the information originally compiled, and the use of derivation procedures that consider all of the compiled information (see also Long and MacDonald 1992; MacDonald 1994; CCME 1995; and MacDonald et al. 1996 for more detailed explanations of these modifications).

The NSTP approach primarily relies on the use of field-collected data, in which chemical mixtures occur, to derive sediment quality assessment values (Long & Morgan 1990; Long 1992; Long and MacDonald 1992; MacDonald 1994; CCME 1995; Long et al. 1995). Data for individual studies are evaluated to establish an *association* between the concentration of each of the chemicals measured in the sediments and any adverse biological effect observed. Cause-and-effect relationships between the concentration of individual chemicals and the observed adverse biological effect cannot be inferred from this evaluation.

Other sediment chemical and biological data may also be used, including models of equilibrium partitioning in sediments, sediment quality assessment values from other jurisdictions (e.g., those derived using the apparent effects threshold approach and the screening level concentration approach), spiked-sediment toxicity tests, and field studies (including acute and chronic toxicity results from sediment bioassays and analyses of benthic community composition). Candidate studies are critically evaluated according to a number of screening criteria to ensure that high quality data sets are used. These screening criteria (e.g., appropriate sediment handling procedures, acceptable toxicity test procedures) have been described in more detail elsewhere (MacDonald 1994; CCME 1995; Long et al. 1995; Environment Canada 1996).

Acceptable data is sorted by sediment type (i.e., freshwater versus marine) and arranged in ascending order of the chemical's concentration to produce separate **ascending data tables**, or **guideline derivation tables**, for individual chemicals and sediment types (CCME 1995; MacDonald 1994; Long et al. 1995). These tables summarize the compiled information that associates a chemical's concentration with either adverse effects or no adverse effects on aquatic organisms (also referred to as the effect data set and the no-effect data set, respectively). Concentrations are expressed as the total concentrations of chemicals in sediments on a dry-weight basis. Many of the previous Canadian sediment quality guidelines were derived based on ascending data tables produced from a large central database called the Biological Effects Database for Sediments (BEDS). However, the guideline derivation tables for nonylphenol did not originate from the BEDS database, but instead were independently compiled.

The guideline derivation tables for individual chemicals (e.g., nonylphenol; Tables 13 and 14) consist of a number of entries, each of which belongs to either the **effect data set** (concentrations associated with an adverse biological effect) or the **no-effect data set** (concentrations not

associated with an adverse biological effect), that are sorted according to ascending chemical concentrations. Entries in the guideline derivation tables were designated as being associated with an effect (an asterisk in the 'Hit' column) if an adverse biological effect was reported. These effects included acute or chronic toxicity observed during a controlled spiked-sediment test, apparent effect thresholds (concentrations above which specific biological effects would always be expected), and predicted toxicity based on equilibrium partitioning theory (which determines a sediment chemical concentration that ensures the concentration of the chemical in the interstitial water does not exceed the water quality guideline for that chemical at equilibrium). An entry was also assigned an asterisk (effect descriptor) if concordance was apparent between the observed biological response and the measured chemical concentration in a field (i.e., co-occurrence) study. Concentrations of individual chemicals reported in field studies were considered to be associated with the observed toxic response (i.e., concordance was apparent) if the mean concentration at sites at which significant adverse effects were observed was a factor of two or more greater than the mean concentration at sites at which effects were not observed (i.e., at toxic versus non toxic sites) (Long et al. 1995). A factor of two was chosen to ensure that the difference in the response (i.e., adverse effect) was associated with a significant difference in the chemical concentration. For each chemical, all of the entries designated by an asterisk, as described above, are collectively referred to as the **effect data set**.

All entries in the guideline derivation tables other than those designated by an asterisk are collectively referred to as the **no-effect data set**, and are represented by those entries for which chemical concentrations were not associated with adverse biological effects. These entries include those associated with non-toxic, reference, or control conditions (i.e., no effects; NE).

Individual entries in the guideline derivation tables consist of the chemical concentration in the sediment, an indication of whether this concentration was part of the effect data set (i.e., *) or the no-effect data set (i.e., NE), location of the study, analysis type (or approach used), test duration, toxicological end-point measured, species and life stage tested, information on the characteristics of the sediments if available (e.g., concentrations of TOC, physical classification of the sediments), and the study reference. In most cases, information for individual entries (i.e., chemical concentrations and sediment chemical and physical characteristics) represents the means of several samples. Standard deviations of these means are provided wherever possible. The following discussion summarizes the information contained in the freshwater and marine guideline derivation tables for nonylphenol (Tables 13 and 14).

Guideline Derivation Tables for Nonylphenol

The freshwater guideline derivation table for nonylphenol (Table 13) contains 25 entries, all of which are results from spiked-sediment toxicity tests (SSTT). The freshwater sediment-associated nonylphenol concentrations in the table range from 2.34 to 680 $mg \cdot kg^{-1}$ dw. A number of adverse biological effects are associated with nonylphenol concentrations ranging from 34.2 to 680 $mg \cdot kg^{-1}$ dw.

Chironomids (*Chironomus tentans*) and frogs (*Rana catesbiana*) are the only freshwater taxa for which toxicological information was obtained and included in the freshwater guideline derivation table. Effects on survival and growth are the most common indicators of adverse biological effects measured for sediment bioassays with laboratory-spiked sediments. Other chronic toxicological endpoints tested include lethargy, haemorrhaging, and tail lesions.

Information on total organic carbon (TOC), pH of the sediment and/or overlying water column, water temperature, conductivity, chemical oxygen demand (COD), biological oxygen demand (BOD), cation exchange capacity (CEC), and the physical classifications of the bed sediments is also included for each entry in the freshwater guideline derivation table, when available (Table 13). The mean TOC content of sediments ranges from 0.05 to 1.27%. The mean proportions of sand, silt, and clay in the freshwater sediments represented in the ascending data table range from 26.4 to 99.4%, <0.7 to 52%, and <0.7 to 21.6%, respectively.

The marine guideline derivation table (Table 14) contains 23 entries, all of which are results from spiked-sediment toxicity tests (SSTT). Concentrations of sediment-associated nonylphenol contained in the table range from 14 to 243 $mg \cdot kg^{-1}$ dw (Table 14). Adverse biological effects associated with nonylphenol in marine sediments occur at concentrations of 98.7 to 243 $mg \cdot kg^{-1}$ dw.

Only two taxa, the amphipod *Ampelisca abdita* and the polychaete *Capitella* sp., are represented in the marine guideline derivation table. Most of the observed adverse biological effects were measurements of various reproductive effects, both at the individual and population level. Size of the organisms at maturity and survival were other endpoints examined.

Information on total organic carbon (TOC), temperature, and salinity were also included for each entry in the marine guideline derivation table (Table 14). The mean TOC content of sediments was 7.4%. The grain size composition of the sediments was not reported in either study.

Derivation of TELs and PELs

As is described in CCME (1995), the derivation procedures used to calculate two assessment values in the original NSTP approach (Long and Morgan 1990) were modified to consider both the effect and no-effect data compiled in the guideline derivation tables. In the modified NSTP approach, the lower value, referred to as the **threshold effect level** (TEL), represents the concentration below which adverse biological effects are expected to occur rarely. The upper value, referred to as the **probable effect level** (PEL), defines the level above which adverse effects are expected to occur frequently. Concentrations that fall in the range between the TEL and the PEL are occasionally expected to be associated with adverse biological effects. The definition of these ranges (also referred to as the minimal, possible, and probable effect ranges) is based on the assumption that the potential for observing toxicity of sediment-associated chemicals increases with increasing chemical concentrations (Long et al. 1995).

Minimum toxicological data requirements have been set to ensure that the TELs calculated from the guideline derivation tables provide adequate protection of aquatic life and that these values are supported by a weight of evidence for a broad range of sediment types and characteristics. For a given guideline derivation table, both the effect data set and the no-effect data set must contain at least 20 entries (CCME 1995). In the case of nonylphenol, the freshwater guideline derivation table contained 8 entries in the effect data set and 17 entries in the no-effect data set. For marine sediments, there were 9 entries in the effect data set and 14 entries in the no-effect data set. Therefore, both the freshwater and marine datasets contained insufficient data to derive TELs or PELs for nonylphenol. To derive a freshwater sediment quality guideline using the NSTP approach, 12 more entries in the effect data set and 3 more entries in the no-effect data set would be required. For derivation of a marine sediment quality guideline using the NSTP approach, 11 more entries in the effect data set and 6 more entries in the no-effect data set would be required to meet the minimum data requirements.

The Spiked-Sediment Toxicity Test (SSTT) Approach

The SSTT approach, which involves an independent evaluation of information from spiked-sediment toxicity tests (that are included in the guideline derivation tables described above), is a complementary procedure to the modified NSTP approach for estimating the concentration of a chemical in sediments below which adverse effects are not expected to occur (CCME 1995). In contrast to the modified NSTP approach, which is used to derive TELs and PELs using information on *associations* between chemical concentrations in sediments and effects on sediment-associated organisms, the SSTT approach is used to derive SSTT values using data from controlled laboratory tests in which organisms are exposed to sediments that have been spiked with known concentrations of a chemical. Such studies provide quantifiable cause-and-effect relationships between the concentration of a chemical in sediments and the observed biological response (e.g., survival, reproductive success, growth). Spiked-sediment toxicity tests are generally used to evaluate the toxic effect of a single chemical, or a specific mixture of chemicals, to exposed organisms. They may also be used to determine the extent to which environmental conditions modify the bioavailability of a chemical, and ultimately the response of organisms exposed to the spiked-sediments.

Data from spiked-sediment toxicity tests must be evaluated and determined to be acceptable before it can be used in the SSTT approach (CCME 1995). In acceptable studies, information on the analytical chemistry of the sediments and overlying water (particularly characteristics that might influence bioavailability of sediment-associated chemicals) should be reported for both the beginning and end of the test. Variables that should be measured in the overlying water include pH, dissolved oxygen, total suspended solids, suspended and dissolved organic carbon, and water hardness or salinity. In the sediment, reported variables should include total organic carbon, particle size distribution, acid volatile sulphide, pH, redox conditions, and sediment type. Generally accepted laboratory practices of exposure and environmental controls should be employed and tests that follow standardized guides or protocols are preferable. Information on the health and survival of the test species for at least a week prior to the test, and repeated evaluation of the condition of the test species throughout the test, should be reported. Where significant mortalities have occurred prior to the test period, the data should not be used. Concentrations of the chemical in sediment must be measured – nominal concentrations are not acceptable. Also, appropriate statistical procedures should be used and reported in detail. Further discussion of data evaluation in provided in the protocol (CCME 1995).

Minimum toxicological data requirements have been set for the SSTT approach to ensure that SSTT values derived using spiked-sediment toxicity information provide adequate protection to aquatic organisms (CCME 1995). Data requirements include at least four independent studies on two or more sediment-resident invertebrate species that occur in North American waters. For freshwater sediments, at least one species must be a benthic crustacean and one must be a benthic arthropod (other than a crustacean). For marine sediments, at least one species must be a benthic amphipod. SSTT values can be derived from studies conducted on sensitive species (e.g., fish, aquatic plants, protozoa, fungi, bacteria) provided that the minimum data set requirements are met. An additional requirement is that at least two of the studies must be partial or full life cycle tests that consider ecologically-relevant endpoints (e.g., growth, reproductive success, developmental effects). In addition to these requirements, the procedures used to generate spiked-sediment toxicity data must be evaluated and determined to be appropriate before they can be used to develop SSTT values. Although methods (e.g., spiking procedures, equilibration periods) for spiked-sediment toxicity tests are currently not standardized (Environment Canada 1995), information provided in individual studies (e.g., equilibrium time, stability of concentrations over the test duration, responses in control treatments) can be used to evaluate the influence of the use of specific methods on test results.

Two independent studies have examined the toxicity of nonylphenol to North American freshwater organisms in spiked-sediment toxicity tests (Appendix IIIa). These studies evaluated nonylphenol toxicity (lethality) to the midge, *Chironomus tentans* (England and Bussard 1993), and to the

amphibian, *Rana catesbiana* (Ward and Boeri 1992). England and Bussard (1993) also assessed sub-lethal endpoints (i.e., growth and reproduction) for *Chironomus tentans*, and Ward and Boeri (1992) examined sub-lethal endpoints for *Rana catesbiana* such as growth, lethargy, haemorrhaging, and occurrence of tail lesions. With only two independent studies, insufficient data are available to derive an SSTT value for freshwater sediments. At least three additional independent studies on North American freshwater sediment-resident invertebrates, at least one of which is a benthic amphipod, are required. Also, at least one of the studies must be a partial or full life-cycle test that reports ecologically relevant sub-lethal endpoints (e.g., growth, reproduction, developmental effects) in order to fulfill the minimum data requirements for the derivation of a freshwater SSTT value.

Two independent studies examined the toxicity of nonylphenol to a marine organism in spiked-sediment toxicity tests (Appendix IIIb). One study evaluated nonylphenol toxicity (lethality) to the amphipod *Ampelisca abdita* (Fay et al. 2000). The other study evaluated sub-lethal effects of nonylphenol on the polychaete *Capitella* sp. (Hansen et al. 1999). Sub-lethal endpoints examined included time to first reproduction, time between reproductive events, number of broods per reproductive individual, number of eggs per individual and per reproductive individual, population growth rate, and size at maturity. These data, however, are insufficient to derive an SSTT value for marine sediments. At least two additional independent studies on North American marine sediment-resident invertebrates are required. Also, at least one of the studies must be a partial or full life-cycle test that reports ecologically relevant sub-lethal endpoints (e.g., growth, reproduction, developmental effects) in order to fulfill the minimum data requirements for the derivation of a marine SSTT value.

Adoption of Guidelines from Other Jurisdictions

When insufficient information is available to derive ISQGs using either the modified NSTP or SSTT approaches, sediment quality assessment values from other jurisdictions are evaluated and, if appropriate, adopted provisionally as ISQGs (CCME 1995). This adoption process gives preference to biological-effects based sediment quality assessment values that have been derived using methods that are philosophically consistent with the protocol's guiding principles. Guidelines that have been derived using effects-based approaches are considered to be the most ecologically relevant and scientifically defensible. Using this approach, the lowest of the guidelines that incorporate data on the effects of sediment-associated substances on sediment-dwelling organisms is selected. For organic contaminants, such as nonylphenol, guidelines derived using the equilibrium partitioning approach can also be considered for adoption.

No sediment quality guidelines for NP or NPEs, either for freshwater or marine sediments, currently exist in any other jurisdiction. Therefore, the approach of adopting sediment guidelines from another jurisdiction cannot be used to develop Canadian sediment quality guidelines.

Table 13. Summary of the available biological effects and related physicochemical data for

NP ($mg \cdot kg^{-1}$)	Hit	Analysis Type	Test Type	Endpoint Measured	Species	Life Stage	TOC (%)	pH sediment
2.34	NE	SSTT	14d	not toxic (7% mortality)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
2.34	NE	SSTT	14d	not toxic (13% growth increase)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
4.79	NE	SSTT	14d	not toxic (11% mortality)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
4.79	NE	SSTT	14d	not toxic (10% growth increase)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
9.51	NE	SSTT	14d	not toxic (8% mortality)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
9.51	NE	SSTT	14d	not toxic (13% growth increase)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
20.1	NE	SSTT	14d	not toxic (10% mortality)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
20.1	NE	SSTT	14d	not toxic (6% growth reduction)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
34.2	*	SSTT	14d	toxic (31% mortality)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
34.2	*	SSTT	14d	toxic (27% growth reduction)	*Chironomus tentans* (freshwater midge)	larvae	1.27	5.5
36	NE	SSTT	30d	not toxic (0% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
36	NE	SSTT	30d	not toxic (5% sublethal effects)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
36	NE	SSTT	30d	not toxic (6% growth reduction)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
57	NE	SSTT	30d	not toxic (0% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
57	NE	SSTT	30d	not toxic (0% sublethal effects)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
57	NE	SSTT	30d	not toxic (7.5% growth reduction)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
155	NE	SSTT	30d	not toxic (10% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
155	NE	SSTT	30d	not toxic (10% sublethal effects)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
155	NE	SSTT	30d	not toxic (25% growth reduction)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
220	*	SSTT	30d	toxic (sublethal effects and growth)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
260	*	SSTT	30d	toxic (50% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
390	*	SSTT	30d	toxic (85% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
390	*	SSTT	30d	toxic (100% sublethal effects - lethargy, haemorrhaging, tail	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
390	*	SSTT	30d	toxic (29% growth reduction)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR
680	*	SSTT	30d	toxic (100% mortality)	*Rana catesbiana* (bullfrog)	tadpole	0.05	NR

TOC = total organic carbon
Temp. = temperature
Cond. = conductivity measured in $\mu Mhos \cdot cm^{-1}$
COD = chemical oxygen demand
BOD = biological oxygen demand
CEC = cation exchange capacity
NE = no effect
* = effect
d = days
NR = not reported

sediment-associated nonylphenol ($mg \cdot kg^{-1}$ dry weight) in freshwater ecosystems.

pH water	Temp. (°C)	Cond.	COD ($mg \cdot L^{-1}$)	BOD ($mg \cdot L^{-1}$)	CEC ($meq \cdot L^{-1}$)	Sand (%)	Silt (%)	Clay (%)	Area	Reference
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
7.2-8.5	19-21	320	352.5	9.2	23.9	26.4	52	21.6	Laboratory	England and Bussard 1993
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992
6.9-8.3	17.1-18.8	740	620 µg/g	45 µg/g	2.3 meq/100g	99.4	<0.7	<0.7	Laboratory	Ward and Boeri 1992

Table 14. Summary of the available biological effects and related physicochemical data for

NP ($mg \cdot kg^{-1}$)	Hit	Analysis Type	Test Type	Endpoint Measured	Species	Life Stage	TOC (%)	pH sediment
14	NE	SSTT	78d	not toxic (1% decrease in time to first reproduction)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (7% increase in time between reproductive events)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (2% decrease in number of broods per reproductive	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (12% increase in number of eggs per reproductive	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (25% decrease in number of eggs per individual)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (11% increase in size at maturity)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
14	NE	SSTT	78d	not toxic (6% increase in population growth rate)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (0% change in time to first reproduction)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (3% increase in time between reproductive events)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (2% increase in number of broods per reproductive	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (3% decrease in number of eggs per reproductive	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (3% decrease in number of eggs per individual)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (16% decrease in size at maturity)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
52	NE	SSTT	78d	not toxic (0% change in population growth rate)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
98.7	*	SSTT	10d	toxic (50% mortality)	*Ampelisca abdita* (amphipod)	juvenile	NR	NR
174	*	SSTT	78d	toxic (17% increase in time to first reproduction)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (46% increase in time between reproductive events)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (44% decrease in number of broods per reproductive individual)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (78% decrease in number of eggs per reproductive individual)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (76% decrease in number of eggs per individual)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (33% decrease in size at maturity)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
174	*	SSTT	78d	toxic (24% decrease in population growth rate)	*Capitella* sp. I (polychaete)	larvae to adult	7.4	NR
243	*	SSTT	10d	toxic (100% mortality)	*Ampelisca abdita* (amphipod)	juvenile	NR	NR

TOC = total organic carbon
Temp. = temperature
NE = no effect
* = effect
d = days
NR = not reported

sediment-associated nonylphenol (mg·kg^{-1} dry weight) in marine ecosystems.

Temp. (°C)	Water Salinity (g·l^{-1})	Sand (%)	Silt (%)	Clay (%)	Area	Reference
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	29-32	NR	NR	NR	Laboratory	Fay et al. 2000
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	26-27	NR	NR	NR	Laboratory	Hansen et al. 1999
20	29-32	NR	NR	NR	Laboratory	Fay et al. 2000

APPENDIX I

Toxicity of nonylphenol and its ethoxylates to freshwater life through water column exposure

APPENDIX 1a: Toxicity of nonylphenol to freshwater algae and macrophytes through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard. ness**	Temp. (°C)	Evaluation Rank	Reference
Ceratophyllum demersum (coontail)	NR	21d	S, N	bleaching	EC_{100}	6250	6.8	NR	NR	23	U	Weinberger and Greenhalgh 1984
Chlamydomonas reinhardii (flagellate algae)	NR	1h	S, N	membrane disruption	NR	10	NR	NR	NR	22	U	Weinberger & Rea 1981
	NR	1h	S, NR	inhibition of photosynthesis	ICF_{100}	500-750	6.7	NR	NR	23	U	Moody et al. 1983
	NR	21d	S, N	growth (biomass)	LOEC	1000	NR	NR	NR	23	U	Weinberger & Greenhalgh 1984
	NR	21d	S, N	algistasis	LOEC	6250-1000	NR	NR	NR	23	U	Weinberger & Greenhalgh 1984
	9d old culture	24h	S, N	ATP reduction	EC_{75} EC_{20}	6250 2500	NR	NR	NR	23	U	Weinberger & Greenhalgh 1984
Chlamydomonas segnis (algae)	4 life cycle phases	24h	S, N	algistatic or algicidal	NR	2500	6.8	NR	NR	23	2	Weinberger et al. 1987
	NR	24h	NR,NR	mortality	LC_{50}	1500	NR	NR	NR	NR	U	Weinberger 1984[1]
	NR	24h	NR,NR	inhibition of photosynthesis	NR	500-750	NR	NR	NR	NR	U	Weinberger 1984[1]
Chlorella pyrenoidosa (algae)	NR	24h	S, N	mortality	LC_{50} LC_{100}	1500 25000	6.8	NR	NR	22	U	Weinberger & Rea 1981, 1982
Lemna minor (duckweed)	2-4 fronds	4d	R, N	growth (dry matter production)	LOEC	125	NR	NR	NR	20	U	Prasad 1989
	2-4 fronds	6d	S, N	growth (frond production)	LOEC	500	NR	NR	NR	20	U	Prasad 1989
	2-4 fronds	4d	S, N	growth (dry matter production)	LOEC	500	NR	NR	NR	20	U	Prasad 1989
	NR	7d	S, N	growth (new frond production)	LOEC	1250	5.6	NR	NR	25	U	Weinberger and Iyengar 1983
	NR	7d	S, N	growth (fresh and dry mass)	LOEC	>1250 >2500	5.6	NR	NR	25	U	Weinberger and Iyengar 1983
	NR	7d	S, N	chlorophyll content	LOEC	1600	5.6	NR	NR	25	U	Weinberger and Iyengar 1983
	2-frond stage	96h	F, M	frond production	NOEC LOEC MATC	901 2080 1369	8.0-8.7	6.5-8.5	108-210	22.8-24.2	1	Brooke 1993a
	NR	7d	S, N	photosynthesis (CO_2 fixation)	LOEC	2500	5.6	NR	NR	25	U	Weinberger and Iyengar 1983

APPENDIX 1a (continued): Toxicity of nonylphenol to freshwater algae and macrophytes through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Lemna minor (duckweed)	NR	24h	S, N	ATP content measurement	LOEC	2500	5.6	NR	NR	25	U	Weinberger and Iyengar 1983
	NR	24h	NR, NR	total ATP	LC_{50}	2500	NR	NR	NR	NR	U	Weinberger 1984[1]
	NR	96h	S, N	growth inhibition	EC_{50}	5500	5.6	NR	NR	25	U	Weinberger and Iyengar 1983
	NR	21d	S, N	bleaching	EC_{100}	6250	6.8	NR	NR	23	U	Weinberger and Greenhalgh 1984
Potamogeton sp. and *Chara* sp. primarily (macrophytes)	NR	21d	R, M	survival	NOEC LOEC MATC	243 >243 >243	7.7	7.3	122	19.5	1	Liber et al. 1999b
Salvinia molesta (aquatic fern)	2-4 fronds	9d	S, N	growth (frond production)	LOEC	2500	NR	NR	NR	20	U	Prasad 1989
Scenedesmus subspicatus (green algae)	NR	72h	NR,NR	growth	EC_{50}	1300	NR	NR	NR	NR	U	Hüls 1996[2]
Selenastrum capricornutum (green algae)	4-7d	96h	S, M	growth (cell counts)	EC_{50} NOEC LOEC MATC	410 92 190 132	7.4-8.9	NR	NR	23.2-23.7	1	Ward & Boeri 1990a
	NR	96h	S, M	cell production	NOEC LOEC MATC	694 1480 1013	7.3-7.7	NR	16	25.2-26.0	1	Brooke 1993a

NR = not reported
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable
ICF_{100} = concentration causing 100% inhibition of fluorescence response

[1] As cited in Servos et al. 2000
[2] As cited in OECD 1997 (as cited in Servos et al. 2000)

APPENDIX Ib. Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Crustacea												
Acanthocyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Acroperus sp. (cladoceran)	NR	92d	R, M	abundance	NOEC	23	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	76						
Alona sp (Cladocera)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Bosmina sp. (cladoceran)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Calanoida (copepod)	nauplii to adult	92d	R, M	abundance	NOEC	5	7.8	8.97	118	17.3	U	O'Halloran et al. 1999; Liber et al. 1999b
					LOEC	23						
					MATC	11						
Ceriodaphnia dubia (water flea)	NEO	7d	R, N	survival	NOEC	3	8.4-8.8	6.9-8.0 (85-101% saturation)	134 - 166	24-25	2	England 1995
					LOEC	10						
					MATC	5.5						
	NEO	7d	R, N	total adverse effects of mortality and delayed maturity	EC_{50}	27	8.4-8.8	6.9-8.0 (85-101% saturation)	134 - 166	24-25	1	England 1995
	NR	96h	F, NR	NR	EC_{50}	69	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	7d	NR,NR	NR	EC_{50}	99	NR	NR	NR	NR	U	Weeks et al. 1996
	NEO	7d	R, N	reproduction (time to first brood, # young/ adult reprod. days	NOEC	100	8.4-8.8	6.9-8.0 (85-101% saturation)	134 - 166	24-25	1	England 1995
					LOEC	300						
					MATC	170						
	NR	7d	F, NR	reproduction	MATC	134	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	7d	NR,NR	mortality	LC_{50}	258	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	96h	NR,NR	mortality	LC_{50}	276	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	7d	NR,NR	NR	MATC	276	NR	NR	NR	NR	U	Weeks et al. 1996)
	NEO	7d	R, N	mortality	LC_{50}	>300	8.4-8.8	6.9-8.0 (85-101% saturation)	134 - 166	24-25	2	England 1995

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Ceriodaphnia dubia (water flea)	NEO	96h	R, N	mortality, total adverse effects	LC_{50} EC_{50}	both >300	8.4-8.8	6.9-8.0 (85-101% saturation)	134 - 166	24-25	2	England 1995
	NR	48h	S, N	mortality	LC_{50}	470 (mean of 340, 710, 430, 410)	8	NR	NR	25	U	Ankley et al. 1990
Ceriodaphnia sp. (water flea)	NR	92d	R, M	abundance	NOEC LOEC	23 76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Chydorus sp. (Cladocera)	NR	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Cladocera (taxa combined)	NR	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Copepoda (taxa combined)	nauplii to adult	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Daphnia galeata mendotae (cladoceran zooplankton)	NEO	30d	R, N	deformity	LOEC	10	NR	NR	NR	20	2	Shurin & Dodson 1997
	NEO	30d	R, N	sex determination (more female offspring)	LOEC	50	NR	NR	NR	20	U	Shurin & Dodson 1997
Daphnia magna (water flea)	NEO	21d	R, M	reproduction	NOEC	24	8.25	NR	180	20	1	Comber et al. 1993
	NR	chronic	NR, NR	reproduction rate	NOEC LOEC MATC	24 39 31	NR	NR	NR	NR	U	Naylor 1995
	NEO	21d	R, M	length of parent	NOEC	39	8.25	NR	180	20	1	Comber et al. 1993
	NEO	21d	R, N	fecundity	MATC	71	8	NR	NR	22	2	Baldwin et al. 1997
	NR	48h	NR, NR	acute toxicity	NOEC LOEC MATC	77 160 110	NR	NR	NR	NR	U	Naylor 1995
	NEO	48h	R, M	mortality	EC_{50}	84.8	7.66-8.04	6.3-8.2	175.6-195.1	21-23	1	Brooke 1993a
	10d adult females	48h	S, N	inc. androgen (from inhibition of testost'one metab. elim.)	LOEC	100	8	NR	NR	22	U	Baldwin et al. 1997
	NR	21d	NR,NR	reproduction	NOEC	100	NR	NR	NR	NR	U	Hüls 1992[2]

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Daphnia magna (water flea)	NEO	21d	R, M	reproduction	NOEC LOEC MATC	116 215 157.9	8.14-8.65	7.5-9.6	156-188	19.7-21.8	1	Brooke 1993a
	NEO	21d	R, M	survival of parent (mortality)	LC_{50}	100	8.25	NR	180	20	1	Comber et al. 1993
	9-10d adult females	96h	R, N	immobilization or mortality	NOEC	0.617 µM (136 $\mu g \cdot L^{-1}$)	7.8-8.4	>60% saturation	227	20	2	Gerritsen et al. 1998
	NR	48h	NR,NR	mortality	LC_{50}	140	NR	NR	NR	NR	U	Hüls 1992[2]
	NR	48h	S, N	immobilization	EC_{50}	180	NR	NR	NR	NR	U	Bringmann and Kühn 1982
	NEO	48h	NR, NR	mortality	LC_{50}	190	NR	NR	NR	20 or 25	U	Naylor 1995
	NEO	48h	S, M	immobilization	EC_{50}	190	8.25	NR	180	20	1	Comber et al. 1993
	NEO	96h	R, N	immobilization or mortality	NOEC	1.37 µM (302 $\mu g \cdot L^{-1}$)	7.8-8.4	>60% saturation	227	20	2	Gerritsen et al. 1998
	NR	48h	NR,NR	mortality	LC_{50}	440	NR	NR	NR	NR	U	Monsanto 1985b[1]
	NEO	48h	NR, NR	reproduction	EC_{50}	440	NR	NR	NR	20 or 25	U	Naylor 1995
	NEO	48h	S, N	immobilization	EC_{50} NOEC LOEC	440 250 500	7.5-8.7	6.7-8.4	264-320	22.1	U	Calvert & Adams 1981
Daphnia pulex (water flea)	JUV	48h	S, N	mortality	LC_{50}	140, 176, 190	NR	NR	27.1	23-27	U	Ernst et al. 1980
Diacyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Eucyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Harpacticoida (copepod)	nauplii to adult	92d	R, M	abundance	NOEC LOEC	76 243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
Hyalella azteca (amphipod)	JUV (2 mm)	96h	F, M	mortality	LC_{50}	20.7	7.69-8.01	7.6-8.8	46-60	23.7-25.6	1	Brooke 1993a
	JUV (2 mm)	96h	F, M	immobilization	EC_{50}	20.7	7.69-8.01	7.6-8.8	46-60	23.7-25.6	1	Brooke 1993a
	NR	96h	F, NR	NR	EC_{50} MATC	150 123	NR	NR	NR	NR	U	Weeks et al. 1996
	JUV	96h	F, M	lethargy and immobility	EC_{50}	150	7.9 - 8.7	1.4 - 8.0	152 - 158	21	1	England & Bussard 1995
	JUV	96h	F, M	mortality	LC_{50}	170	7.9 - 8.7	1.4 - 8.0	152 - 158	21	1	England & Bussard 1995

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Kurzia sp. (cladoceran)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Macrocyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Mesocyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Ostracoda (ostracod)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Paracyclops sp. (copepod)	nauplii to adult	92d	R, M	abundance	NOEC	5	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	23						
Pleuroxus sp. (Cladocera)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Simocephalus sp. (cladoceran)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Insecta												
Chironomini (chironomid)	NR	21d	R, M	survival	NOEC	243	7.7	7.3	122	19.5	1	Liber et al. 1999b; Schmude et al. 1999
					LOEC	>243						
					MATC	>243						
Chironomus tentans (freshwater midge)	LAR (24h to 4th instar)	20d	R, M	survival	NOEC	42	7.73	7.04	NR	23	1	Kahl et al. 1997
					LOEC	91						
	LAR-ADT	53d	R, M	emergence, reproduction, sex ratio	NOEC	45	7.73	7.04	NR	23	2	Kahl et al. 1997
	LAR (2nd instar)	14d	F, M	total adverse eff. (lethargy, small size, pale)	EC_{50}	95	7.6-8.6	0.5-7.5	130-154	19-21	1	England and Bussard 1993
	LAR (2nd instar)	14d	F, M	larval weight (growth)	EC_{50}	95	7.6-8.6	0.5-7.5	130-154	19-21	1	England and Bussard 1993
					LOEC	150						
					NOEC	76						
					MATC	107						
	LAR (2nd instar)	14d	F, M	mortality (survival)	LC_{50}	119	7.6-8.6	0.5-7.5	130-154	19-21	1	England & Bussard 1993
					LOEC	150						
					NOEC	76						
					MATC	107						
	LAR	96h	NR,NR	mortality	LC_{50}	119	NR	NR	NR	NR	U	Weeks et al. 1996, Naylor 1995
					MATC	107						
	NR	96h	F, NR	NR	EC_{50}	160	NR	NR	NR	NR	U	Weeks et al. 1996
					MATC	97						

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Ophiogomphus sp. (dragonfly)	NYM (285 mg)	96h	F, M	loss of equilibrium, posterior of abdomen flexed over back	EC_{50}	596	7.82-8.52	7.7-8.9	50.7-54.6	21.5-25.0	1	Brooke 1993a
	NYM (285 mg)	96h	F, M	mortality	LC_{50}	>768	7.82-8.52	7.7-8.9	50.7-54.6	21.5-25.0	1	Brooke 1993a
Tanytarsini (chironomid)	LAR	21d	R, M	survival	NOEC LOEC	76 243	7.7	7.3	122	19.5	U	Schmude et al. 1999
Mollusca												
Anodonta cataractae (freshwater clam)	15g	144h	R, M	mortality	LC_{50}	1700	NR	NR	NR	10	U	McLeese et al. 1980a
Elliptio complanata (mussel)	excised gills	50 min	S, N	metabolic heat rate inhibition	LOEC	70000	NR	NR	NR	30	U	Levine and Cheney 2000
	excised gills	50 min	S, N	respiratory inhibition	LOEC	220300	NR	NR	NR	25	U	Levine and Cheney 2000
Gastropoda (snails, mostly Planorbidae)	NR	21d	R, M	survival	NOEC LOEC	76 243	7.7	7.3	122	19.5	U	Schmude et al. 1999
Physella virgata (snail)	ADT (476 mg)	96h	F, M	mortality	LC_{50}	774	7.73-8.02	7.8-8.2	50-60	22.8-24.5	1	Brooke 1993a
	ADT (476 mg)	96h	F, M	unable to retract into shell with prodding	EC_{50}	378	7.73-8.02	7.8-8.2	50-60	22.8-24.5	1	Brooke 1993a
Pisidium sp. (bivalve)	NR	21d	R, M	survival	NOEC LOEC MATC	23 76 42	7.7	7.3	122	19.5	U	Schmude et al. 1999; Liber et al. 1999b
Oligochaeta												
Naididae (oligochaete)	NR	21d	R, M	survival	NOEC LOEC	23 76	7.7	7.3	122	19.5	U	Schmude et al. 1999
Tubificidae (oligochaete)	NR	21d	R, M	survival	NOEC LOEC	243 >243	7.7	7.3	122	19.5	U	Schmude et al. 1999
Annelida												
Lumbriculus variegatus (annelid)	ADT (5 mg)	96h	F, M	mortality	LC_{50}	342	6.63-6.9	7.2-8.5	50.7-54.6	22.2-25.9	1	Brooke 1993a
	ADT (5 mg)	96h	F, M	inactivity	EC_{50}	268	6.63-6.9	7.2-8.5	50.7-54.6	22.2-25.9	1	Brooke 1993a

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Rotifera												
Rotifera (taxa combined)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999; Liber et al. 1999b
					LOEC	>243						
					MATC	>243						
Brachionus sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Colurella sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Conochilus sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Euchlanis sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Keratella sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Lecane sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Lepadella sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Monostyla sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Mytilina sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Notholca sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Platyias sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Pleosoma sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Polyarthra sp. (rotifer)	NR	92d	R, M	abundance	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	>243						
Testudinella sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						
Trichocerca sp. (rotifer)	NR	92d	R, M	abundance	NOEC	23	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	76						
Trichotria sp. (rotifer)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999
					LOEC	243						

APPENDIX Ib (continued). Toxicity of nonylphenol to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Miscellaneous												
Macroinvertebrates (taxa combined)	NR	21d	R, M	survival	NOEC	76	7.7	7.3	122	19.5	U	Liber et al. 1999b
					LOEC	243						
					MATC	136						
Periphyton (taxa combined)	NR	92d	R, M	survival	NOEC	243	7.8	8.97	118	17.3	U	O'Halloran et al. 1999; Liber et al. 1999b
					LOEC	>243						
					MATC	>243						
Tetrahymena pyriformis (freshwater protozoan)	NR	24h	S, N	relative growth rate	EC_{50}	460	NR	NR	NR	30	U	Yoshioka et al. 1985
Zooplankton (taxa combined)	NR	92d	R, M	abundance	NOEC	76	7.8	8.97	118	17.3	U	O'Halloran et al. 1999; Liber et al. 1999b
					LOEC	243						
					MATC	136						

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, PUP = pupae, LAR = larvae, NYM = nymph, NEO = neonates
* duration reported in minutes (min), hours (h), or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

[1] As cited in Etnier 1985 as cited in Talmage 1994
[2] As cited in Servos et al. 2000

APPENDIX Ic. Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Culaea inconstans (5-7-spined stickleback)	NR	NR	21d	R, M	survival	NOEC LOEC MATC	243 >243 >243	7.7	7.3	122	19.5	1	Liber et al. 1999b
Gasterosteus aculeatus (stickleback)	NR	NR	96h	NR,NR	mortality	LC_{50}	370	NR	NR	NR	NR	U	Granmo et al. 1991a
Gila elegans (bonytail chub)	0.29-0.52 g	NR	96h	S, N	mortality	LC_{50}	290	8.35	low values	173	22	2	Dwyer et al. 1995
Ictalurus punctatus (channel catfish)	JUV (65-95g)	NR	7d	IPS, N	vitellogenesis	LOEC	237 $mg \cdot kg^{-1}$ (1)	NR	NR	NR	NR	U	Nimrod & Benson 1996
	hepato-cytes from adult fish 400-500g	male	4-6d	R, N	vitellogenin induction	LOEC	10 µM (2203 $\mu g \cdot L^{-1}$)	NR	NR	NR	NR	2	Monteverdi and Di Giulio 1999
						NOEC	1 µM (220 $\mu g \cdot L^{-1}$)						
	hepato-cytes from 2 yr old fish (300-400g)	female	5h	S, NR	inhibition of estradiol binding to estrogen receptor site	IC_{50}	4531 nM (998 $\mu g \cdot L^{-1}$)	NR	NR	NR	room temp	U	Nimrod and Benson 1997
Lebistes reticulatus (guppy)	NR	NR	24h	F, NR	NR	NR	NR	NR	NR	NR	NR	U	Swedmark 1968
Lepomis macrochirus (bluegill sunfish)	11-13 wks old	NR	28d	F, M	mortality	NOEC LOEC	59.5 126	6.80-7.78	4.4-7.4	46.8-51.2	23.3-26.0	1	Brooke 1993b
	JUV (9 cm, 12 g)	NR	21d	R, M	survival	NOEC LOEC MATC	76 243 136	7.7	7.3	122	19.5	1	Liber et al. 1999a
	13-15 wks old	NR	96h	F, M	mortality	NOEC LOEC	86.5 211	7.51-7.99	5.2-8.0	49.4-50.8	25.3-26.6	1	Brooke 1993b
	JUV (<1 year old), 23 mm, 294 mg	NR	96h	F, M	loss of equilibrium, loss of schooling ability	EC_{50}	203	7.26-7.79	7.5-8.2	50.7-54.6	21.5-23.8	1	Brooke 1993a

APPENDIX Ic (continued). Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hardness**	Temp. (°C)	Evaluation Rank	Reference
Lepomis macrochirus (bluegill sunfish)	JUV (<1 yr old), 23 mm, 294 mg	NR	96h	F, M	mortality	LC_{50}	209	7.26-7.79	7.5-8.2	50.7-54.6	21.5-23.8	1	Brooke 1993a
Leuciscus idus (golden orfe)	NR	NR	48h	NR,NR	mortality	LC_{50}	950	NR	NR	NR	NR	U	Hüls 1987
Oncorhynchus apache (Apache trout)	0.38-0.85 g	NR	96h	S, N	mortality	LC_{50}	170	8.24	low values	169	12	2	Dwyer et al. 1995
Oncorhynchus clarki henshawi (Lahontan cutthroat trout)	0.31 g	NR	96h	S, N	mortality	LC_{50}	180, 170	8.24	low values	169	12	2	Dwyer et al. 1995
Oncorhynchus clarki stomias (greenback cutthroat trout)	0.34-0.57 g	NR	96h	S, N	mortality	LC_{50}	150, 156	8.24	low values	169	12	2	Dwyer et al. 1995
Oncorhynchus mykiss (rainbow trout)	JUV	female	22d,35d	F, N	growth inhibition	LOEC	1	6.5	NR	12.5	7-13	U	Ashfield et al. 1998
	eggs	male	12 mo.	NR,NR	vitellogenin induction	LOEC	1	NR	NR	NR	NR	U	Fent et al. 1999
	eggs	male	12 mo.	NR,NR	Vg mRNA induction	LOEC	1	NR	NR	NR	NR	U	Fent et al. 1999
	EMB	**NR**	**91d**	**F, M**	**growth**	**NOEC** **LOEC** **MATC**	**6** **10.3** **7.861**	**6.34-7.84**	**7.7-11.7**	**39.0-66.3**	**8.9-14.4**	**1**	**Brooke 1993a**
	ADT and JUV	male and female	72h	F, N	vitellogenin induction	LOEC	10	NR	NR	NR	10-15	U	Lech et al. 1996
	ADT and JUV	male and female	72h	F, N (calc)	estrogenic (vitellogenin induction)	EC_{50}	14.14	NR	NR	NR	10-15	U	Lech et al. 1996
	2 yr	male	21d	F, M	elevation of plasma vitellogenin	LOEC	20.3	NR	NR	NR	NR	2	Jobling et al. 1996
	hepatocytes	male	24h	S, N	Vg mRNA induction; rtER mRNA induction	NOEC LOEC	10^{-7} M ($22 \mu g \cdot L^{-1}$) 10^{-6} M ($220 \mu g \cdot L^{-1}$)	NR	NR	NR	NR	U	Flouriot et al. 1995

APPENDIX Ic (continued). Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oncorhynchus mykiss (rainbow trout)	JUV (sexually immature 35-50g)	NR	21d	R, N	vitellogenin induction	NOEL LOEL	25 50	8.0-8.4	>90% satur-ation	NR	13	2	Tremblay and Van Der Kraak 1998
	2 yr	male	21d	F, M	500 fold increase in vitellogenin	NR	37	NR	NR	NR	NR	U	Jobling et al. 1996
	JUV (46-174g)	NR	9d	IPS, N	vitellogenin induction	NOEC	50 $mg \cdot kg^{-1}$	NR	NR	NR	NR	U	Andersen et al. 1999
	JUV (103-168g)	NR	12d	IPR, NR	vitellogenin induction	LOEC (only conc tested)	50 $mg \cdot kg^{-1}$	NR	NR	NR	NR	U	Pedersen et al. 1999
	JUV (103-168g)	NR	12d	IPR, NR	vitellogenin induction	NOEC (only conc tested)	50 $mg \cdot kg^{-1}$	NR	NR	NR	NR	U	Pedersen et al. 1999
	JUV (100-200g)	NR	NR	F, N	induction of vitellogenin mRNA	significant effect	100	NR	NR	NR	10-15	U	Ren et al. 1996
	45d, 27.2 mm, 241 mg	NR	96h	F, M	loss of equilibrium, poor fright response	EC_{50}	109	6.53-6.98	9.3-10.4	54.6-58.5	11.3-12.6	1	Brooke 1993a
	JUV (103-168g)	NR	9d	F, M	vitellogenin induction	LOEC	75	NR	NR	NR	NR	U	Pedersen et al. 1999
	JUV (103-168g)	NR	9d	F, M	vitellogenin induction	NOEC	109	NR	NR	NR	NR	U	Pedersen et al. 1999
	0.27-1.25 g	NR	96h	S, N	mortality	LC_{50}	190, 250	8.24	low values	169	12	2	Dwyer et al. 1995
	ADT and JUV (50-200g)	male and female	72h	F, N	mortality	LC_{50}	193.65	NR	NR	NR	10-15	U	Lech et al. 1996
	45d, 27.2 mm, 241 mg	NR	96h	F, M	mortality	LC_{50}	221	6.53-6.98	9.3-10.4	54.6-58.5	11.3-12.6	1	Brooke 1993a
	JUV	NR	96h	NR,NR	mortality	LC_{50}	230	NR	NR	NR	NR	U	Armstrong and Kingsbury 1979[2]

APPENDIX Ic (continued). Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oncorhynchus mykiss (rainbow trout)	42-60mm, 0.5-2.7g	NR	96h	S, N	mortality	LC_{50}	230	NR	NR	NR	9	U	Holmes and Kingsbury 1980
	15-30d	NR	96h	NR,NR	mortality	LC_{50}	230	NR	>6	NR	12	U	Naylor 1995
	EMB	NR	acute-24h exp + 48h post-exp	S, M	mortality	LC_{50}	484	NR	NR	27.1	9.5-28.5	U	Ernst et al. 1980
	JUV (1.4g, 5.2 cm)	NR	96h	S, N	mortality	LC_{50}	920, 560	6.2-8.3	4.6-10.6	27.1	15-16	U	Ernst et al. 1980
	hepato-cytes from JUV fish	males	48h	S, N	vitellogenin production	ED_{50}	16.15 µM (3558 $\mu g \cdot L^{-1}$)	NR	NR	NR	NR	U	Jobling and Sumpter 1993
Oryzias latipes (Japanese medaka)	5-8d old, larval swim-up stage	NR	28d (& 1 mo. in clean water)	F, M	sex ratio, fecundity, egg viability or hatchability	NOEC	1.93	NR	NR	NR	25	2	Nimrod and Benson 1998
	hatch to 3 mths	NR	3 mo.	R, N	induction of testis-ova	NOEC LOEC	10 50	7.4-7.8	NR	80-100	20	U	Gray & Metcalfe 1997
	hatch to 3 mths	NR	3 mo.	R, N	sex ratio	NOEC LOEC	50 100	7.4-7.8	NR	80-100	20	U	Gray & Metcalfe 1997
	EMB	NR	17d	S, N	embryotoxicity	LC_{50}	460	NR	NR	NR	25	U	Gray and Metcalfe 1997
	2 cm, 0.2 g	NR	48h	S, NR	mortality	LC_{50}	1400	7.2	NR	50	16.2	U	Yoshimura 1986
Pimephales promelas (fathead minnow)	ADT	NR	42d	F, M	histologic lesions	LOEC NOEC	1.6 - 3.4 0.4 - 0.93	NR	NR	NR	25-26	2	Miles-Richardson et al. 1999
	EMB	NR	33d	F, M	survival	NOEL LOEL MATC	7.4 14 10.2	7.1-8.2	6.3-9.7	160-180	23.4-26.3	1	Ward and Boeri 1991b
	early life stage	NR	30d	F, NR	NR	MATC	10.2	NR	NR	NR	NR	U	Weeks et al. 1996
	EMB	NR	33d	F, M	# embryos hatched, time to hatch, time to first feeding, total length, weight	 NOEC LOEC MATC	for all endpoints: 23 >23 >23	7.1-8.2	6.3-9.7	160-180	23.4-26.3	1	Ward and Boeri 1991b

APPENDIX Ic (continued). Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Pimephales promelas (fathead minnow)	4 wks old	NR	28d	F, M	mortality	NOEC LOEC	77.5 193	6.91-7.92	4.4-7.7	45.8-52.2	23.2-26.3	1	Brooke 1993b
	4 wks old	NR	96h	F, M	mortality	NOEC LOEC	83.1 230	7.50-7.84	5.0-6.7	46.2-48.4	23.0-25.6	1	Brooke 1993b
	25-35d old, 81.6g, 17.1 mm	NR	96h	F, M	loss of equilibrium, poor schooling and fright responses	EC_{50}	96	6.77-7.58	6.6-8.2	53.6-63.4	21.3-23.1	1	Brooke 1993a
	31-35d, 220 mg	NR	96h	F, M	loss of equilibrium	LOEC	98	6.9-7.7	7.4	44.9	24.6	2	Holcombe et al. 1984
	25-35d old, 81.6g, 17.1 mm	NR	96h	F, M	mortality	LC_{50}	128	6.77-7.58	6.6-8.2	53.6-63.4	21.3-23.1	1	Brooke 1993a
	31-35d, 220 mg	NR	96h	F, M	mortality	LC_{50}	135	6.9-7.7	7.4	44.9	24.6	1	Holcombe et al. 1984
	32d	NR	96h	F, M	mortality	LC_{50}	140	7.29	7.1	45.1	24.5	1	Geiger et al. 1985
	31-35d	NR	96h	F, M	haemorr-haging	LOEC	187	6.9-7.7	7.4	44.9	24.6	2	Holcombe et al. 1984
	31-35d	NR	96h	F, M	bodies swollen with fluid	LOEC	187	6.9-7.7	7.4	44.9	24.6	2	Holcombe et al. 1984
	31-35d, 220 mg	NR	96h	F, M	lethargic	LOEC	187	6.9-7.7	7.4	44.9	24.6	2	Holcombe et al. 1984
	0.32-0.56g	NR	96h	S, N	mortality	LC_{50}	270, 320	8.35	low values	173	22	2	Dwyer et al. 1995
	0.21g, 26 mm	NR	96h	S, N	mortality	LC_{50} NOEC	300 180	7	9.2	45	22	2	Analytical BioChemistry Laboratories, Ltd. 1981
Ptychocheilus lucius (Colorado squawfish)	0.32-0.34 g	NR	96h	S, N	mortality	LC_{50}	260, 240	8.35	low values	173	22	2	Dwyer et al. 1995
Salmo salar (Atlantic salmon)	1 yr, immature 23g	NR	30d	IPR, N	smoltification vitellogenesis	sig. effect	3 mg NP in 100µL oil	NR	NR	NR	8-12	U	Madsen et al. 1997
	1 yr, immature23g	NR	30d	IPR, N	osmoregul-ation	sig. effect	3 mg NP in 100µL oil	NR	NR	NR	8-12	U	Madsen et al. 1997

APPENDIX Ic (continued). Toxicity of nonylphenol to freshwater fish through water column exposure

Species (Common name)	Life stage	Sex	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Salvelinus fontinalis (brook trout)	36-55mm, 0.5-1.4g	NR	96h	S, N	mortality	LC_{50}	145	6.5-7.0	NR	51.6 [3]	ambient stream temp.	U	Holmes and Kingsbury 1980
	NR	NR	96h	NR,NR	mortality	LC_{50}	145	NR	NR	NR	NR	U	Armstrong and Kingsbury 1979 [1]
Salvelinus namaycush Walbaum (lake trout)	54-92mm, 0.6-60g	NR	35d	S, N	mortality	LC_{50}	>40	NR	NR	NR	12.5	U	Holmes and Kingsbury 1980
Xiphophorus maculatus (platyfish)	ADT 0.6-1.2 g	males	28d	R, N	survival	NOEC LOEC	320 640	8.1	NR	NR	28	U	Kinnberg et al. 2000
	ADT 0.6-1.2 g	males	28d	R, N	growth	NOEC	1280	8.1	NR	NR	28	U	Kinnberg et al. 2000
Xyrauchen texanus (razorback sucker)	0.31-0.32 g	NR	96h	S, N	mortality	LC_{50}	170, 200	8.35	low values	173	22	2	Dwyer et al. 1995

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h), days (d), or months (mo.)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent), IPS = single intraperitoneal injection, IPR = repeated intraperitoneal injections
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable
Critical study for freshwater Canadian Water Quality Guideline appears in bold

[1] Units are in $mg \cdot kg^{-1}$ because the nonylphenol was administered as an agar suspension that was injected intraperitoneally
[2] As cited in McLeese et al. 1981
[3] In Holmes and Kingsbury (1980) this value was given as 3 GPG (grains per gallon), but has been converted here to $mg \cdot L^{-1}$ $CaCO_3$ (by multiplying by a factor of 17.2).

APPENDIX Id. Toxicity of nonylphenol ethoxylates to freshwater algae and macrophytes through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Algae													
Scenedesmus sp. (green algae)	NR	NP4EO	NR	NR,NR	mortality	lethal threshold	6000	NR	NR	NR	NR	U	Janicke et al. 1969
Scenedesmus sp. (green algae)	NR	NP6EO	NR	NR,NR	mortality	lethal threshold	10000	NR	NR	NR	NR	U	Janicke et al. 1969
Selenastrum capricornutum (green algae)	NR	NP6EO	21d	S, N	growth	NOEC	500000	NR	NR	NR	25	U	Nyberg 1988
Scenedesmus sp. (green algae)	NR	NP7EO	NR	NR,NR	mortality	lethal threshold	16000	NR	NR	NR	NR	U	Janicke et al. 1969
Selenastrum capricornutum (green algae)	NR	NP7EO	96h	NR,NR	growth	EC_{50}	>1000000	NR	NR	NR	NR	U	Markarian et al. 1989[1], 1990
Selenastrum capricornutum (green algae)	NR	NP8EO	48h	S, N	growth inhibition	EC_{50}	20000	7.4	NR	NR	24	2	Yamane et al. 1984
Selenastrum capricornutum (green algae)	NR	NP9EO	96h	NR,NR	growth	EC_{50}	50000	NR	NR	NR	NR	U	Lewis 1986
	4-7d	NP9EO	96h	S, M	cell count (growth)	EC_{50} NOEC LOEC MATC	12000 8000 16000 11300	7-7.5	2-5	150	22-25	1	Dorn et al. 1993
	NR	NP9EO	21d	S, N	growth	NOEC	500000	NR	NR	NR	25	U	Nyberg 1988
	NR	NP9EO	48h	S, N	growth inhibition	EC_{50}	50000	7.4	NR	NR	24	2	Yamane et al. 1984
green and blue-green algae	NR	NP9.5EO	NR	NR,NR	growth inhibition	NR	20000	NR	NR	NR	NR	U	Davis & Gloyna 1967[2]
Scenedesmus sp. (green algae)	NR	NP10EO	NR	NR,NR	mortality	lethal threshold	31000	NR	NR	NR	NR	U	Janicke et al. 1969
Scenedesmus sp. (green algae)	NR	NP20EO	NR	NR,NR	mortality	lethal threshold	125000	NR	NR	NR	NR	U	Janicke et al. 1969

APPENDIX Id (continued). Toxicity of nonylphenol ethoxylates to freshwater algae and macrophytes through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Selenastrum capricornutum Printz (green algae)	NR	NP30EO	21d	S, N	growth	NOEC	500000	NR	NR	NR	25	U	Nyberg 1988
Scenedesmus sp. (green algae)	NR	NP30EO	NR	NR,NR	mortality	lethal threshold	5000000	NR	NR	NR	NR	U	Janicke et al. 1969
Macrophytes													
Egeria densa (macrophyte)	leaflets removed from plants	NP4EO	several hours	S, NR	depolarization of membrane potential	LOEC	10000	7.3	NR	NR	NR	U	Linsel et al. 1990
Egeria densa (macrophyte)	leaflets removed from plants	NP20EO	several hours	S, NR	depolarization of membrane potential	LOEC	100000	7.3	NR	NR	NR	U	Linsel et al. 1990

NR = not reported
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

[1] As cited in Servos et al. 2000
[2] As cited in Talmage 1994

APPENDIX Ie. Toxicity of nonylphenol ethoxylates to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Crustacea													
Hyalella azteca (amphipod)	2-4d	mixture of APEs	96h	S, N	mortality	LC_{50}	450	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	0-2d	mixture of APEs	96h	S, N	mortality	LC_{50}	600	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	6-8d	mixture of APEs	96h	S, N	mortality	LC_{50}	750	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	4-6d	mixture of APEs	96h	S, N	mortality	LC_{50}	850	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	16-18d	mixture of APEs	96h	S, N	mortality	LC_{50}	870	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	10-12d	mixture of APEs	96h	S, N	mortality	LC_{50}	900	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	20-22d	mixture of APEs	96h	S, N	mortality	LC_{50}	1250	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
	24-26d	mixture of APEs	96h	S, N	mortality	LC_{50}	1520	7.4-8.1	6.3-8.2	40	25	U	Collyard et al. 1994
Ceriodaphnia dubia (water flea)	NEO	NP1.5EO	7d	R, M	survival and reproduction	NOEC LOEC MATC	285 886 503	8.0 - 8.9	4.0-8.5 (49-106% saturation)	140 - 154	23-25	1	England & Downing 1995
	NEO	NP1.5EO	7d	R, M	total adverse effects	EC_{50}	319	8.0 - 8.9	4.0-8.5 (49-106% saturation)	140 - 154	23-25	1	England & Downing 1995
	NR	NP1.5EO	7d	NR,NR	NR	EC_{50}	319	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	NP1.5EO	7d	NR,NR	reproduction	MATC	503	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	NP1.5EO	96h	NR,NR	NR	EC_{50}	626	NR	NR	NR	NR	U	Weeks et al. 1996
	NEO	NP1.5EO	96h	R, M	total adverse effects - mortality, small size, pale appearance	EC_{50}	626	8.0 - 8.9	4.0-8.5 (49-106% saturation)	140 - 154	23-25	1	England & Downing 1995
	NEO	NP1.5EO	7d	R, M	mortality	LC_{50}	886	8.0 - 8.9	4.0-8.5 (49-106% saturation)	140 - 154	23-25	1	England & Downing 1995

APPENDIX Ie (continued). Toxicity of nonylphenol ethoxylates to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Ceriodaphnia dubia (water flea)	NR	NP1.5EO	7d	NR,NR	mortality	LC_{50}	886	NR	NR	NR	NR	U	Weeks et al. 1996
	NEO	NP1.5EO	96h	R, M	mortality	LC_{50}	1016	8.0 - 8.9	4.0-8.5 (49-106% saturation)	140 - 154	23-25	1	England & Downing 1995
	NR	NP1.5EO	96h	NR,NR	mortality	LC_{50}	1016	NR	NR	NR	NR	U	Weeks et al. 1996
	NR	NP1.5EO	48h	S, N	mortality	LC_{50}	1040	8	NR	NR	25	2	Ankley et al. 1990
Daphnia magna (water flea)	NEO	NP2EO	48h	S, NNR	mortality	LC_{50}	67	NR	NR	hard	20	U	Maki et al. 1998
	NEO	NP2EO	48h	S, NR	mortality	LC_{50}	148	NR	NR	hard	20	U	Maki et al. 1998
Daphnia magna (water flea)	NR	NP7EO	96h	NR,NR	mortality	LC_{50}	4100					U	Markarian et al. 1989[1]
Daphnia magna (water flea)	NEO	NP9EO	7d	R, M	mortality	LC_{50} NOEC LOEC MATC	9000 10000 20000 14000	7-7.5	2-5	150	22-25	1	Dorn et al. 1993
	NEO	NP9EO (Igepal CO-630, GAF Corp.)	7d	R, M	carapace length (growth)	NOEC LOEC MATC	10000 >10000 >10000	7-7.5	2-5	150	22-25	1	Dorn et al. 1993 (also Kravetz et al. 1991)
	NEO	NP9EO	48h	R, M	mortality	LC_{50}	14000	7-7.5	2-5	150	25	1	Dorn et al. 1993 (also Kravetz et al. 1991)
Daphnia pulex (water flea)	NR	NP9EO	48h	NR,NR	immobiliz-ation	EC_{50}	2870	NR	NR	NR	NR	U	Salanitro et al. 1988
Daphnia magna (water flea)	NR	NP10EO	24h	NR,NR	mortality	LC_{50}	44200	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[2]
Daphnia pulex (water flea)	NR	NP10EO	48h	S, NR	mortality	LC_{50}	12520	7.0-8.0	3.7-7.5	25-40	20-21	U	Moore et al. 1987
Daphnia magna (water flea)	NEO	NPnEO	21d	R, N	fecundity	NOEC	5000	8.0	NR	NR	22		Baldwin et al. 1998

APPENDIX Ie (continued). Toxicity of nonylphenol ethoxylates to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Daphnia magna (water flea)	NEO	NPnEO	21d, then 16h with testost-erone	R, N	metabolic elimination of testosterone	EC_{100}	5000	8.0	NR	NR	20	U	Baldwin et al. 1998
	10d females	NPnEO	64h (16 with testost-erone)	S, N	metabolic elimination of testosterone	NOEC	5000	8.0	NR	NR	22	U	Baldwin et al. 1998
	NEO	NPnEO	21d	R, N	reproduction	chronic value	5700	8.0	NR	NR	22	U	Baldwin et al. 1998
	NEO	NPnEO	48h	NR, N	mortality	LC_{50}	8600	8.0	NR	NR	22	U	Baldwin et al. 1998
Daphnia magna (water flea)	NR	NP1EC	48h	NR,NR	mortality	LC_{50}	14000	NR	NR	NR	NR	U	Naylor et al. 1997
						NOEC	11000						
Ceriodaphnia dubia (water flea)	NR	NP1EC	7d	NR,NR	reproduction	NOEC	2200	NR	NR	NR	NR	U	Naylor et al. 1997
	NR	NP1EC	7d	NR,NR	mortality	NOEC	8400	NR	NR	NR	NR	U	Naylor et al. 1997
	NR	NP1EC	96h	NR,NR	mortality	LC_{50}	17000	NR	NR	NR	NR	U	Naylor et al. 1997
Daphnia magna (water flea)	NEO	NP2EC	48h	S, NR	mortality	LC_{50}	141	NR	NR	hard	20	U	Maki et al. 1998
	NEO	NP2EC	48h	S, NR	mortality	LC_{50}	990	NR	NR	hard	20	U	Maki et al. 1998
Insects													
Culex pipiens (southern house mosquito)	PUP	NP1EO	NR	S, NR	emergence	EC_{50}	80000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP1.5EO	NR	S, NR	emergence	EC_{50}	67000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP4EO	NR	S, NR	emergence	EC_{50}	6000 - 8000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP6EO	NR	S, NR	emergence	EC_{50}	6000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968

APPENDIX Ie (continued). Toxicity of nonylphenol ethoxylates to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Culex pipiens (mosquito)	PUP	NP9EO	NR	S, NR	emergence	EC_{50}	10000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP9.5EO	NR	S, NR	emergence	EC_{50}	7000 - 19000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP10EO	NR	S, NR	emergence	EC_{50}	19000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Aëdes aegypti (mosquito)	LAR (4th stage)	NP11EO	10d	NR, NR	pupae mortality	LC_{50}	50000	NR	NR	NR	25	U	van Emden et al. 1974
	LAR (2nd & 3rd stage)	NP11EO	24h	NR, NR	mortality	LC_{50} LC_{100}	500000 1000000	NR	NR	NR	25	U	van Emden et al. 1974
	EGG	NP11EO	10d	NR, NR	hatch rate	EC_{50}	500000	NR	NR	NR	25	U	van Emden et al. 1974
Culex pipiens (mosquito)	PUP	NP15EO	NR	S, NR	emergence	EC_{50}	9000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP20EO	NR	S, NR	emergence	EC_{50}	28000, 120000, >200000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP30EO	NR	S, NR	emergence	EC_{50}	>400000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968
Culex pipiens (mosquito)	PUP	NP50EO	NR	S, NR	emergence	EC_{50}	>400000	7.5-8.0	NR	NR	78°F	U	Maxwell and Piper 1968

APPENDIX Ie (continued). Toxicity of nonylphenol ethoxylates to freshwater invertebrates through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Mollusca													
Elliptio complanata (mussel)	excised gills	NP1.5EO	50 min	S, N	metabolic heat rate inhibition	LOEC	9000	NR	NR	NR	30	U	Levine and Cheney 2000
Biomphalaria glabrata (snail)	1.5-2 cm	NP11EO	24h	NR, NR	mortality	LC_{100}	23000	NR	NR	NR	25	U	van Emden et al. 1974
Cnidaria													
Hydra littoralis (hydra)	mixture of budded, unbudded	NPnEO	11d	R, NR	growth	threshold concentra-tion	640	NR	NR	NR	NR	U	Stephenson 1979

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, PUP = pupae, LAR = larvae, NYM = nymph, NEO = neonates
* duration reported in minutes (min), hours (h), or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

[1] As cited in Servos et al. 2000
[2] As cited in Anonymous 1977 as cited in Talmage 1994

APPENDIX If. Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP1EO	48h	S, NR	mortality	LC_{50}	3000	7.2	NR	50	16.2	U	Yoshimura 1986
Oryzias latipes (Japanese medaka)	1 day post-hatch	NP1.5EO	90d	R, N	growth	NOEC	100	NR	NR	NR	22-24	2	Metcalfe et al. 2001
	1 day post-hatch	NP1.5EO	90d	R, N	sex ratio	NOEC	100	NR	NR	NR	22-24	2	Metcalfe et al. 2001
	1 day post-hatch	NP1.5EO	90d	R, N	testis-ova	NOEC LOEC	50 100	NR	NR	NR	22-24	U	Metcalfe et al. 2001
Oncorhynchus mykiss (rainbow trout)	JUV (females)	NP2EO	22d,35d	F, N	growth inhibition	LOEC	1	6.5	NR	12.5	7-13	U	Ashfield et al. 1998
	2 yr-old males	NP2EO	21d	F, M	200 fold increase in vitellogenin	NR	38	NR	NR	NR	NR	U	Jobling et al. 1996
	hepato-cytes from JUV males	NP2EO	48h	S, N	vitellogenin production	ED_{50}	17.27 µM (5326 $\mu g \cdot L^{-1}$)	NR	NR	NR	NR	U	Jobling and Sumpter 1993
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP3.3EO	48h	S, NR	mortality	LC_{50}	2500	7.2	NR	50	16.2	U	Yoshimura 1986
Lepomis macrochirus (bluegill sunfish)	NR	NP4EO	96h	S, M	mortality	LC_{50}	1300	7.1	9.0-5.1	NR	18	U	Macek and Krzeminski 1975
Lepomis macrochirus (bluegill sunfish)	NR	NP5EO	96h	S, M	mortality	LC_{50}	2400 - 2800	7.1	9.0-5.1	NR	18	U	Macek and Krzeminski 1975
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP5EO	48h	S, NR	mortality	LC_{50}	3600	7.2	NR	50	16.2	U	Yoshimura 1986
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP6.4EO	48h	S, NR	mortality	LC_{50}	5400	7.2	NR	50	16.2	U	Yoshimura 1986
Pimephales promelas (fathead minnows)	NR	NP7EO	48h	NR,NR	mortality	LC_{50}	3200	NR	NR	NR	NR	U	Markarian et al. 1989[1]

APPENDIX If (continued). Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oncorhynchus mykiss (rainbow trout)	12-16 cm	NP8EO	14d	F, N	mortality	LC_{50}	4250	7.3-7.4	>70% saturation	290-310	15-15.6	U	Calamari and Marchetti 1973
	12-16 cm	NP8EO	96h	F, N	mortality	LC_{50}	4700	7.3-7.4	>70% saturation	290-310	15-15.6	U	Calamari and Marchetti 1973
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP8.4EO	48h	S, NR	mortality	LC_{50}	11600	7.2	NR	50	16.2	U	Yoshimura 1986
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP8.9EO	48h	S, NR	mortality	LC_{50}	11200	7.2	NR	50	16.2	U	Yoshimura 1986
Carassius auratus (goldfish)	NR	NP9EO	48h	NR,NR	mortality	LC_{50}	18000	NR	NR	NR	NR	U	Tomiyama 1974
Lepomis macrochirus (bluegill sunfish)	NR	NP9EO	192h	F, N	mortality	LC_{50}	6300	7.1	9.4-8.9	38	21	U	Macek and Krzeminski 1975
	NR	NP9EO	96h	S, M	mortality	LC_{50}	7900	7.1	9.0-5.1	NR	18	U	Macek and Krzeminski 1975
Leuciscus idus (golden orfe)	NR	NP9EO	48h	NR,NR	mortality	LC_{50}	7000	NR	NR	NR	NR	U	Fischer 1973[2]
Oncorhynchus mykiss (rainbow trout)	hepato-cytes from JUV males	NP9EO	48h	S, N	vitellogenin production	ED_{50}	82.31 µM (50800 $\mu g \cdot L^{-1}$)	NR	NR	NR	NR	U	Jobling and Sumpter 1993
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP9EO	48h	S, NR	mortality	LC_{50}	12000	7	NR	3400	16.2	U	Yoshimura 1986
	2 cm, 0.2 g	NP9EO	48h	S, NR	mortality	LC_{50}	14000	7	NR	25	16.2	U	Yoshimura 1986
Pimephales promelas (fathead minnow)	1d	NP9EO	7d	R, M	weight at 7d (growth)	NOEC LOEC MATC	1000 2000 1400	7-7.5	2-5	150	22-25	1	Dorn et al. 1993 (also Kravetz et al. 1991)
	NR	NP9EO	96h	NR,NR	mortality	LC_{50}	1620	NR	NR	NR	NR	U	Salanitro et al. 1988
	1d	NP9EO	7d	R, M	mortality	LC_{50} NOEC LOEC MATC	2900 1800 2000 1400	7-7.5	2-5	150	22-25	1	Dorn et al. 1993
	7-27d	NP9EO	96h	R, M	mortality	LC_{50}	4600	7-7.5	2-5	150	22-25	1	Dorn et al. 1993
	NR	NP9EO	96h	NR,NR	mortality	LC_{50}	6600	NR	NR	NR	NR	U	Williams et al. 1996[1]

APPENDIX If (continued). Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Carassius aurtus (goldfish)	5-6 cm	NP9.5EO	6h	S, NR	mortality	LC_{50}	6900	NR	NR	100	20	U	Reiff et al. 1979
Rasbora heteromorpha (harlequin fish)	1.3-3.0 cm	NP9.5EO	96h	F, NR	mortality	LC_{50}	8600	NR	NR	20	20	U	Reiff et al. 1979
Lepomis macrochirus (bluegill sunfish)	NR	NP9.5EO	96h	S, M	mortality	LC_{50}	7600	7.1	9.0-5.1	NR	18	U	Macek and Krzeminski 1975
Leuciscus idus[4] (golden orfe)	5-7 cm	NP9.5EO	48h	S, NR	mortality	LC_{50}	4900	NR	NR	150	20	U	Reiff et al. 1979
	5-7 cm	NP9.5EO	96h	F, NR	mortality	LC_{50}	7000	NR	NR	268	20	U	Reiff et al. 1979
	5-7 cm	NP9.5EO	96h	S, NR	mortality	LC_{50}	11200	NR	NR	268	20	U	Reiff et al. 1979
Oncorhynchus mykiss (rainbow trout)	1 yr old, 9.4 cm, 8.3g	NP9.5EO	24h	S, NR	loss of equilibrium	EC_{50}	2800	7.5-8.1	>90% satur-ation	114	15	U	Muller 1980
	NR	NP9.5EO	96h	NR,NR	mortality	LC_{50}	7500-12500 4500-5900	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]
	1 yr old, 9.4 cm, 8.3g	NP9.5EO	24h	S, NR	mortality	LC_{50}	8500	7.5-8.1	>90% satur-ation	114	15	U	Muller 1980
Salmo trutta (brown trout)	2.8 or 5.8 cm	NP9.5EO	96h	F, NR	mortality	LC_{50}	1000	NR	NR	26-30	15	U	Reiff et al. 1979
Carassius auratus (goldfish)	5cm	NP10EO	48h	NR, N	mortality	LC_{50}	5400	NR	NR	NR	NR	U	Kurata et al. 1977
	NR	NP10EO	48h	NR,NR	mortality	LC_{50}	13800	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]
Rasbora heteromorpha (harlequin fish)	NR	NP10EO	48h	NR,NR	mortality	LC_{50}	11300	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]
Leuciscus idus (golden orfe)	NR	NP10EO	48h	NR,NR	mortality	LC_{50}	7400, 9500	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]
Oncorhynchus mykiss (rainbow trout)	perfused head of fish 160-220 g	NP10EO	1h	F, N	change in gill resistence; sodium net flux	NOEC LOEC	87.6 (+0.9 Cd) 63000 (+905 Cd)	7	meas-ured	NR	10	U	Pärt et al. 1985

APPENDIX If (continued). Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oncorhynchus mykiss (rainbow trout)	23 days after hatching	NP10EO	6h	S, N	mortality	LC_{50}	2100	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	fry 25 days after hatching	NP10EO	6h	S, N	mortality	LC_{50}	2300	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	40d old (fry)	NP10EO	6h	S, N	mortality	LC_{50}	5200	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	210d old (finger-ling)	NP10EO	6h	S, N	mortality	LC_{50}	5200	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	alevin 12 days after hatching	NP10EO	6h	S, N	mortality	LC_{50}	5200	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	alevin 19 days after hatching	NP10EO	6h	S, N	mortality	LC_{50}	5200	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	3-10 cm	NP10EO	96h	NR,NR	mortality	LC_{50}	7500-11700	NR	NR	20-300	10 and 15	U	Unilever Research Laboratories 1977[3]
	alevin 6 days after hatching	NP10EO	6h	S, N	mortality	LC_{50}	22000	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	alevin immedi-ately after hatching	NP10EO	6h	S, N	mortality	LC_{50}	42000	7.3-7.4	5	240-260	10-14	U	Marchetti 1965
	perfused head of fish 160-220 g	NP10EO	1h	F, N	change in gill resistence & O_2 transfer factor; Na^+ net flux	significantly different from control	63000	7	meas-ured	NR	10	U	Pärt et al. 1985
Phoxinus phoxinus (minnow)	NR	NP10EO	48h	NR,NR	mortality	LC_{50}	8800	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]
Salmo trutta (brown trout)	NR	NP10EO	48h	NR,NR	mortality	LC_{50}	2700	NR	NR	NR	NR	U	Unilever Research Laboratories 1977[3]

APPENDIX If (continued). Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Lebistes reticulatus (guppy)	14d JUV (0.007g, 0.7cm)	NP11EO	24h	NR, NR	mortality	LC_{100}	52000	NR	NR	NR	25	U	van Emden et al. 1974
Lebistes reticulatus (guppy)	females (0.12-0.38g, 2 cm)	NP11EO	24h	NR, NR	mortality	LC_{100}	57000	NR	NR	NR	25	U	van Emden et al. 1974
Lebistes reticulatus (guppy)	males (0.05-0.08g, 2 cm)	NP11EO	24h	NR, NR	mortality	LC_{100}	64000	NR	NR	NR	25	U	van Emden et al. 1974
Oncorhynchus mykiss (rainbow trout)	JUV (46-174g)	NP12EO	9d	IPS, N	vitellogenin induction	NOEC	50 $mg \cdot kg^{-1}$	NR	NR	NR	NR	U	Andersen et al. 1999
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP13.1EO	48h	S, NR	mortality	LC_{50}	48000	7.2	NR	50	16.2	U	Yoshimura 1986
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	NP16.6EO	48h	S, NR	mortality	LC_{50}	110000	7.2	NR	50	16.2	U	Yoshimura 1986
Lepomis macrochirus (bluegill sunfish)	NR	NP30EO	96h	S, M	mortality	LC_{50}	$>1 \times 10^6$	7.1	9.0-5.1	NR	18	U	Macek and Krzeminski 1975
Pimephales promelas (fathead minnow)	sexually mature	NP7-11EO	42d	F, M	histologic lesions	NOEC	10	NR	NR	NR	25-26	2	Miles-Richardson et al. 1999
Oncorhynchus mykiss (rainbow trout)	JUV (females)	NP1EC	22d,35d	F, N	growth inhibition	LOEC	10	6.5	NR	12.5	7-13	U	Ashfield et al. 1998
	2yr males	NP1EC	21d	F, M	100-fold increase in vitellogenin	NR	32	NR	NR	NR	NR	U	Jobling et al. 1996
	hepato-cytes from immature males	NP1EC	48h	S, N	vitellogenin production	ED_{50}	15.25 µM (4916 $\mu g \cdot L^{-1}$)	NR	NR	NR	NR	U	Jobling and Sumpter 1993
Pimephales promelas (fathead minnow)	NR	NP1EC	96h	NR,NR	mortality	LC_{50} NOEC	2000 1000	NR	NR	NR	NR	U	Williams et al. 1996[1]

APPENDIX If (continued). Toxicity of nonylphenol ethoxylates to freshwater fish through water column exposure

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Hard-ness**	Temp. (°C)	Evaluation Rank	Reference
Oryzias latipes (Japanese medaka)	1d post-hatch	NP1.5EC	90d	R, N	growth	NOEC	100	NR	NR	NR	22-24	2	Metcalfe et al. 2001
	1d post-hatch	NP1.5EC	90d	R, N	sex ratio	NOEC	100	NR	NR	NR	22-24	2	Metcalfe et al. 2001
	1d post-hatch	NP1.5EC	90d	R, N	testis-ova	NOEC	100	NR	NR	NR	22-24	2	Metcalfe et al. 2001
Oryzias latipes (Japanese killifish)	2 cm, 0.2 g	sodium 4-NP2EC	48h	S, NR	mortality	LC_{50}	8900	7.2	NR	50	16.2	U	Yoshimura 1986
	2 cm, 0.2 g	sodium 4-NP1EC	48h	S, NR	mortality	LC_{50}	9600	7.2	NR	50	16.2	U	Yoshimura 1986

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent), IPS = single intraperitoneal injection, IPR = repeated intraperitoneal injections
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
** hardness reported in mg $CaCO_3 \cdot L^{-1}$
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

[1] As cited in Servos et al. 2000
[2] As cited in Gloxhuber 1974
[3] As cited in Anonymous 1977 as cited in Talmage 1994
[4] Although reported as *Idus idus* in the original article, this species has since been renamed as *Leuciscus idus*

APPENDIX II

Toxicity of nonylphenol and its ethoxylates to marine aquatic life through water column exposure

APPENDIX IIa. Toxicity of nonylphenol to marine algae through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Skeletonema costatum (algae)	NR	96h	S, M	growth inhibition (cell count)	EC_{50}	27	7.9-9.6	NR	30	21-22	1	Ward and Boeri 1990d
					NOEC	10						
	NR	96h	F, NR	NR	EC_{50}	27	NR	NR	NR	NR	U	Weeks et al. 1996 (also cited in Naylor 1995)
					NOEC	10						
					LOEC	20						
					MATC	14.1						

NR = not reported
* duration reported in hours (h)
Test type: S = static, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment
Conc. = concentration
D.O. = dissolved oxygen
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

APPENDIX IIb. Toxicity of nonylphenol to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Crustaceans												
Acartia tonsa (calanoid copepod)	ADT (10-12d)	48h	S, N	mortality	LC_{50}	190	NR	NR	18	20	U	Kusk and Wollenberger 1999
					LC_{50}	280						
					LC_{50}	360						
***Americamysis bahia*[1] (mysid)**	**NEO**	**28d**	**F, M**	**mean total length**	**NOEL**	**3.9**	**7.4-8.3**	**5.0-8.5**	**20-21**	**23.2-26.4**	**1**	**Ward and Boeri 1991a**
					LOEL	**6.7**						
					MATC	**5.1**						
	NR	28d	F, NR	length	MATC	5.1	NR	NR	NR	NR	U	Weeks et al. 1996 (also cited in Naylor 1995)
					NOEC	3.9						
					LOEC	6.7						
	NEO	28d	F, M	number of young per surviving female	NOEL	6.7	7.4-8.3	5.0-8.5	20-21	23.2-26.4	1	Ward and Boeri 1991a
					LOEL	9.1						
					MATC	7.8						
	NEO	28d	F, M	survival	NOEL	6.7	7.4-8.3	5.0-8.5	20-21	23.2-26.4	1	Ward and Boeri 1991a
					LOEL	9.1						
					MATC	7.8						
					LC_{50}	>21						
	NR	28d	F, NR	reproduction	MATC	7.8	NR	NR	NR	NR	U	Weeks et al. 1996 (also cited in Naylor 1995)
					NOEC	6.7						
					LOEC	9.1						
	NR	28d	F, NR	survival	MATC	7.8	NR	NR	NR	NR	U	Weeks et al. 1996 (also cited in Naylor 1995)
					NOEC	6.7						
					LOEC	9.1						
	NEO (2.8 mg)	96h	F, M	mortality	LC_{50}	43	7.3-8.2	6.5-7.8	20	23.8-25.3	1	Ward and Boeri 1990b
					NOEC	18						
	NR	96h	F, NR	mortality	LC_{50}	43	NR	NR	NR	NR	U	Weeks et al. 1996 (also cited in Naylor 1995)
	post-larvae 1-day	96h	F, M	mortality	LC_{50}	60.6	7.8-8.2	NR	30-31	24-25	1	Lussier et al. 2000
Balanus amphitrite (barnacle)	LAR	1-6d	R, M	cypris major protein production	50% increase	0.023	NR	NR	NR	28	U	Billinghurst et al. 2000
	LAR (day-3 cypris)	48h	R, M	reduced settlement	LOEC	0.059	NR	NR	NR	28	2	Billinghurst et al. 1998
	LAR	1-6d	R, M	cypris major protein production	2-fold increase	0.693	NR	NR	NR	28	U	Billinghurst et al. 2000

APPENDIX IIb (continued). Toxicity of nonylphenol to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Balanus amphitrite (barnacle)	LAR (day-2 cypris)	48h in NP, 48h in clean water	R, M	reduced settlement	LOEC	0.693	NR	NR	NR	25	U	Billinghurst et al. 1998
Corophium volutator Pallas (amphipod)	<5d JUV mean 1.02 mm	120d	S , N	density and population growth	LOEC	10	NR	NR	25	15	U	Brown et al. 1999
	<5d JUV mean 1.02 mm	120d	S , N	time to maturity; length; sex ratio	NOEC	10	NR	NR	25	15	U	Brown et al. 1999
	mean 4.38 mm	42d	S , N	mortality	96h LC_{50} 10d LC_{50} 30d LC_{50}	1670 620 270	NR	NR	25	15	U	Brown et al. 1999
Crangon crangon (brown shrimp)	NR	7d	NR, NR	mortality	LC_{50}	340	NR	NR	NR	NR	U	Waldock & Thain 1991[2]
	NR	96h	NR, NR	mortality	LC_{50}	420	NR	NR	NR	NR	U	Waldock & Thain 1991[2]
	NR	96h	R,NR	mortality	LC_{50}	600	NR	NR	32	17	U	Granmo et al. 1991a
Crangon septemspinosa (sand shrimp)	2 g each	96h	R, M	mortality	LC_{50}	300	NR	NR	NR	10	U	McLeese et al. 1981
	1.3g	96h	R, M	mortality	LC_{50}	300	NR	NR	NR	10	U	McLeese et al. 1980a
Crassostrea gigas (Pacific oyster)	LAR	72h	S, N	delayed larval development	LOEC	100	measured	NR	35	20	2	Nice et al. 2000
	LAR	48h	S, N	deformity	LOEC	100	measured	NR	35	20	2	Nice et al. 2000
Dyspanopeus sayii (mudcrab)	zoea 4th & 5th stage	96h	F, M	mortality	LC_{50}	>195	7.8-8.2	NR	30-31	24-25	2	Lussier et al. 2000
Homarus americanus (American lobster)	zoea first stage	96h	R, N	mortality	LC_{50}	71	7.8-8.2	NR	30-31	16-17	2	Lussier et al. 2000
	NR (450g)	96h	R, M	mortality	LC_{50}	170	NR	NR	NR	10	U	McLeese et al. 1980a
Leptocheirus plumulosus (amphipod)	ADT	96h	F, M	mortality	LC_{50}	[illegible]1.6	7.8-8.2	NR	30-31	24-25	1	Lussier et al. 2000
Nitocra spinipes (harpacticoid)	NR	96h	NR, NR	mortality	LC_{50}	11[illegible], 139	NR	NR	NR	NR	U	Bergström 1984[3]
Paleomonetes vulgaris (grass shrimp)	LAR (2-day)	96h	F, M	mortality	LC_{50}	59.4	7.8-8.2	NR	30-31	24-25	1	Lussier et al. 2000

APPENDIX IIb (continued). Toxicity of nonylphenol to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Tisbe battagliai (copepod)	nauplii <1 day old	53d	R, M	reduced survival of offspring, intrinsic rate of increase, egg product-ion	NOEC LOEC	20 41	NR	NR	25	10	1	Bechmann 1999
	nauplii <1 day old	96h	S, N	mortality	LC_{50}	31-62	NR	NR	25	10	2	Bechmann 1999
	ADT	96h	S, N	mortality	LC_{50}	500-1000	NR	NR	25	10	2	Bechmann 1999
Molluscs												
Mulinia lateralis (coot clam)	EMB - LAR	48h	S, N	inhibition of fertilization	EC_{50}	37.9	7.8-8.2	NR	32	18.3	2	Lussier et al. 2000
Mya arenaria (soft-shelled clam)	NR	144h	R, M	mortality	NOEC	>700	NR	NR	NR	10	U	McLeese et al. 1980a
	NR (20g)	360h	R, M	mortality	NOEC	>1000	NR	NR	NR	10	U	McLeese et al. 1980a
Mytilus edulis (common blue mussel)	ADT (40-50mm)	30d	F, N	byssus strength	NOEC LOEC	32 56	NR	NR	32	10	U	Granmo et al. 1989
	NR	32d	NR, NR	growth & strength	LOEC	56	NR	NR	NR	NR	U	Naylor 1995
	NR	30d	R,NR	byssus strength	LOEC	56	NR	NR	32	17	U	Granmo et al. 1991a
	NR	850h	R,NR	mortality	LC_{50}	140	NR	NR	32	17	U	Granmo et al. 1991a
	eggs to veligers	72h	NR, N	fertilization, development	NOEC	200	NR	NR	32	17	U	Granmo et al. 1989
	NR	96h	NR, NR	mortality	LC_{50}	2600	NR	NR	NR	NR	U	Naylor 1995
	ADT (40-50mm)	32d	R, N	mortality	96h LC_{50} 360h 850h	3000 500 140	NR	NR	32	17	U	Granmo et al. 1989
	NR	96h	R,NR	mortality	LC_{50}	3000	NR	NR	32	17	U	Granmo et al. 1991a

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent)
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration; D.O. = dissolved oxygen; Temp. = temperature

Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable
Critical study for marine Canadian Water Quality Guideline appears in bold

[1] This species was formerly named *Mysidopsis bahia*
[2] As cited in Servos et al. 2000
[3] As cited in Wahlberg et al. 1990

APPENDIX IIc. Toxicity of nonylphenol to marine fish through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Agonus cataphractus (hook nose)	NR	7d	NR, NR	mortality	LC_{50}	360	NR	NR	NR	NR	U	Waldock and Thain 1991[1]
	NR	96h	NR, NR	mortality	LC_{50}	510	NR	NR	NR	NR	U	Waldock and Thain 1991[1]
Cyprinodon bovinus (Leon Springs pupfish)	NR	96h	NR, NR	NR	LC_{50}	480	NR	NR	NR	NR	U	Brecken-Folse et al. 1995
Cyprinodon variegatus (sheepshead minnow)	JUV	96h	F, M	mortality	LC_{50}	142	7.8-8.2	NR	30-31	24-25	1	Lussier et al. 2000
	JUV (mean 0.55g ww, 26.3 mm)	96h	F, M	mortality; bloating, lethargy, etc.	LC_{50} NOEC	310 240	7.4-8.1	7.0-8.8 (60-105% saturation)	NR	22 (21.4-23.0)	1	Ward and Boeri 1990c
	NR	96h	NR, NR	NR	LC_{50}	460	NR	NR	NR	NR	U	Brecken-Folse et al. 1995
Fundulus heteroclitus (killifish)	LAR (2 weeks old)	96h	R, N	mortality	LC_{50}	209	NR	NR	20	20	2	Kelly and Di Giulio 2000
	EMB	10d	R, N	sublethal abnormalities	LOEC	2203	NR	NR	20	20	2	Kelly and Di Giulio 2000
	EMB	96h	R, N	mortality	LC_{50}	5441	NR	NR	20	20	2	Kelly and Di Giulio 2000
Gadus morhua (cod)	NR	21d	F, NR	mortality	median tolerance limit	<100	NR	NR	NR	16	U	Swedmark 1968
	NR	96h	F, NR	mortality	median tolerance limit	300	NR	NR	NR	16	U	Swedmark 1968
	NR	96h	NR, NR	mortality	LC_{50}	370	NR	NR	32	NR	U	Granmo et al. 1991a
Gasterosteus aculeatus (3-spined stickleback)	NR	96h	R, NR	mortality	LC_{50}	370	NR	NR	32	17	U	Granmo et al. 1991a
Menidia beryllina (inland silversides)	JUV	96h	F, M	mortality	LC_{50}	70	7.8-8.2	NR	30-31	24-25	1	Lussier et al. 2000
Platichthys flesus (flounder)	ADT (male)	15d	IPR, N	dose-dependent increase in vitellogenin observed	NR	10 - 200 $mg \cdot kg^{-1} \cdot week^{-1}$	NR	NR	NR	NR	U	Bjerregaard et al. 1998

APPENDIX IIc (continued). Toxicity of nonylphenol to marine fish through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Platichthys flesus (flounder)	ADT	21d	F, M	vitellogenin induction	NOEC	24.5	NR	>80%	34	10	2	Allen et al. 1999
	ADT	21d	F, M	effect on gonadosoma-tic index	NOEC	24.5	NR	>80%	34	10	2	Allen et al. 1999
	ADT	21d	F, M	increased hepatosoma-tic index	LOEC	24.5	NR	>80%	34	10	2	Allen et al. 1999
Pleuronectes americanus (winter flounder)	LAR (2-day)	96h	S, M	mortality	LC_{50}	17	7.8-8.2	NR	30-31	5	2	Lussier et al. 2000
Salmo salar (Atlantic Salmon)	JUV (8 g each)	96h	F, M	mortality	LC_{50}	130, 160	NR	NR	NR	10	U	McLeese et al. 1981
	JUV (8 g each)	96h	R, M	mortality	LC_{50}	190	NR	NR	NR	10	U	McLeese et al. 1981
	hepatocytes from males or juveniles	48h	R, N	induction of zona radiata proteins	NOEC	1 µM (220.3 $\mu g \cdot L^{-1}$)	NR	NR	NR	10	U	Celius et al. 1999
					LOEC	5 µM (1100 $\mu g \cdot L^{-1}$)						
	JUV (4g)	96h	R, NR	mortality	LC_{50}	900	NR	NR	NR	10	U	McLeese et al. 1980a
	hepatocytes from males or juveniles	96h	R, N	vitellogenin induction	NOEC	5 µM (1100 $\mu g \cdot L^{-1}$)	NR	NR	NR	10	U	Celius et al. 1999
					LOEC	10 µM (2203 $\mu g \cdot L^{-1}$)						
	JUV (ca. 1 year old) 167g	14d	IPS, N	increased vitellogenin and zona radiata protein levels	significant effect	25 $mg \cdot kg^{-1}$	NR	NR	34	10	U	Arukwe et al. 2000a
	JUV (ca. 1 year old) 75-120g	14d	F (water); S (NP), N	decreased EROD activity	LOEC	125 $mg \cdot kg^{-1}$ fish	NR	NR	NR	10	U	Arukwe et al. 1997a
					NOEC	25 $mg \cdot kg^{-1}$ fish						

APPENDIX IIc (continued). Toxicity of nonylphenol to marine fish through water column exposure.

Species (Common name)	Life stage	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Zoarces viviparus (eelpout)	pregnant females (70g)	21d	R, N	increased vitellogenin conc.	LOEC	100	NR	NR	20	11	2	Korsgaard and Pedersen 1998
					NOEC	50						
	EMB	21d	R, N	increased vitellogenin conc.	LOEC	1000	NR	NR	20	NR	U	Korsgaard and Pedersen 1998
	males	21d	R, N	vitellogenin increased 10000-fold	NR	1000	NR	NR	NR	12	U	Christiansen et al. 1998b,c
	ADT (male)	25d	IPR, N	vitellogenin increased 10000-fold	NR	0.1 $mg \cdot g^{-1} \cdot week^{-1}$	NR	NR	NR	12	U	Christiansen et al. 1998b,c
	males	25d	IPR, N	vitellogenin increased 1000-fold	NR	[70.1] $mg \cdot g^{-1} \cdot week^{-1}$	NR	NR	NR	12	U	Christiansen et al. 1998b,c

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent), IPS = single intraperitoneal injection, IPR = repeated intraperitoneal injections
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable
[1] As cited in Servos et al. 2000

APPENDIX IId. Toxicity of nonylphenol ethoxylates to marine algae and macrophytes through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
12 species marine phytoplankton	NR	NP9EO	14d	NR, NR	growth inhibition	EC_{50}	10000	NR	NR	NR	NR	U	Ukeles 1965
	NR	NP30EO	14d	NR, NR	growth inhibition	EC_{50}	1000000	NR	NR	NR	NR	U	Ukeles 1965

NR = not reported
* duration reported in days (d)
Conc. = concentration
D.O. = dissolved oxygen
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

APPENDIX IIe. Toxicity of nonylphenol ethoxylates to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Crustaceans													
Americamysis bahia [1] (mysid)	3-8 days old	NP1.5EO	48h	R, M	mortality	LC_{50}	110	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
Americamysis bahia[1] (mysid)	NR	NP2EO	48h	R, NR	mortality	LC_{50}	ca. 150	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
Americamysis bahia[1] (mysid)	3-8 days old	NP9EO	48h	R, N	mortality	LC_{50}	900 - 2000 710 - 1560	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
	NR	NP9EO	48h	R, NR	mortality	LC_{50}	1230	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
	NR	$NP9EOPO_3^{-2}$	48h	R, NR	mortality	LC_{50}	4600	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
	3-8 days old	NP9EO phosphated	48h	R, N	mortality	LC_{50}	5230	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
	3-8 days old	NP9EO sulphonated	48h	R, N	mortality	LC_{50}	29600 24300	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
	NR	$NP9EOSO_3^-$	48h	R, NR	mortality	LC_{50}	29600	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
Balanus balanoides (common barnacle)	LAR (stage II nauplius)	NP10EO	96h	R, N	mortality	LC_{50}	1500	NR	NR	NR	6-8	U	Swedmark et al. 1971
	ADT	NP10EO	96h	F, N	mortality	LC_{50}	<25000	NR	NR	NR	6-8, 15-17	U	Swedmark et al. 1971
Carcinus maenas. (shore crab)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	>100000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Eupagurus bernhardus (hermit crab)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	>100000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Hyas araneus (spider crab)	LAR (stage 1 zoea)	NP10EO	96h	S, N	mortality	LC_{50}	10000	NR	NR	NR	6-8	U	Swedmark et al. 1971
	ADT	NP10EO	96h	F, N	mortality	LC_{50}	>100000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Leander adspersus (decapod)	postmoult phase	NP10EO	96h	F, N	mortality	LC_{50}	10000	NR	NR	NR	15-17	U	Swedmark et al. 1971
	intermoult phase	NP10EO	96h	F, N	mortality	LC_{50}	50000	NR	NR	NR	15-17	U	Swedmark et al. 1971
	ADT	NP10EO	96h	F, N	mortality	LC_{50}	>100000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Leander squilla (decapod)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	>100000	NR	NR	NR	6-8	U	Swedmark et al. 1971

APPENDIX IIe (continued). Toxicity of nonylphenol ethoxylates to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Crangon crangon (brown shrimp)	NR	NP12EO	48h	NR, NR	mortality	LC_{50}	89500	NR	NR	NR	NR	U	Portmann and Wilson 1971[2]
Carcinus maenus (shore crab)	NR	NP12EO	48h	NR, NR	mortality	LC_{50}	>100000	NR	NR	NR	NR	U	Portmann and Wilson 1971[2]
	NR	NP12EO	48h	NR, NR	mortality	LC_{50}	>100000	NR	NR	NR	NR	U	Waldock and Thain 1991[3]
Pandalus montagui (pink shrimp)	NR	NP12EO	48h	NR, NR	mortality	LC_{50}	19300	NR	NR	NR	NR	U	Portmann and Wilson 1971[2]
Americamysis bahia[1] (mysid)	NR	NP15EO	48h	R, NR	mortality	LC_{50}	ca. 2000	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
	3-8 days old	tp-NP15EO	48h	R, N	mortality	LC_{50}	2570	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
Americamysis bahia[1] (mysid)	3-8 days old	tp-NP40EO	48h	R, N	mortality	LC_{50}	>100000	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
	NR	NP40EO	48h	R, NR	mortality	LC_{50}	ca. 100000	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
Americamysis bahia[1] (mysid)	NR	NP50EO	48h	R, NR	mortality	LC_{50}	ca. 4000000	NR	NR	NR	NR	U	Patoczka and Pulliam 1990
	3-8 days old	tp-NP50EO	48h	R, N	mortality	LC_{50}	>4110000	7.7-8.0	>90% saturation	NR	25	2	Hall et al. 1989
Americamysis bahia[1] (mysid)	NR	NP1EC	96h	NR, NR	mortality	LC_{50} NOEC	9400 4100	NR	NR	NR	NR	U	Naylor et al. 1997
Mollusca													
Monodonta turbiformis (snail)	NR	NP9.5EO	14d	NR, NR	differential survival of allozyme genotypes	NR	7000-117000	8.3	NR	NR	22	U	Lavie et al. 1984
Monodonta turbinata (snail)	NR	NP9.5EO	14d	NR, NR	differential survival of allozyme genotypes	NR	6740-67400	8.3	NR	NR	22	U	Lavie et al. 1984

APPENDIX IIe (continued). Toxicity of nonylphenol ethoxylates to marine invertebrates through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Cardium edule (cockle)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	5000	NR	NR	NR	6-8	U	Swedmark et al. 1971
	JUV	NP10EO	96h	F, N	mortality	LC_{50}	<<10000	NR	NR	NR	15-17	U	Swedmark et al. 1971
Mya arenaria (clam)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	<10000	NR	NR	NR	15-17	U	Swedmark et al. 1971
	ADT	NP10EO	96h	F, N	mortality	LC_{50}	18000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Mytilus edulis (common blue mussel)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	<10000	NR	NR	NR	15-17	U	Swedmark et al. 1971
	ADT	NP10EO	96h	F, N	mortality	LC_{50}	12000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Pecten maximus (scallop)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	<<5000	NR	NR	NR	15-17	U	Swedmark et al. 1971
Pecten opercularis	ADT	NP10EO	96h	F, N	mortality	LC_{50}	<<10000	NR	NR	NR	15-17	U	Swedmark et al. 1971
Cardium edule (cockle)	NR	NP12EO	48h	NR, NR	mortality	LC_{50}	92500	NR	NR	NR	NR	U	Portmann and Wilson 1971[2]

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h) or days (d)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent), IPS = single intraperitoneal injection, IPR = repeated intraperitoneal injections
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration
D.O. = dissolved oxygen
Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable

[1] This species was formerly named *Mysidopsis bahia*
[2] As cited in Talmage 1994
[3] As cited in Servos et al. 2000

APPENDIX IIf. Toxicity of nonylphenol ethoxylates to marine fish through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP5EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	1200	7.92	95-98% saturation	NR	12	2	Miossec and Bocquéné 1986
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP8EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	3000	7.78	94-104% saturation	NR	12	2	Miossec and Bocquéné 1986
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP10EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	3300	7.89	94-95% saturation	NR	12	2	Miossec and Bocquéné 1986
Gadus morhua (cod)	NR	NP10EO	NR	NR, NR	avoidance behaviour		>20	NR	NR	NR	NR	U	Hoglund 1976[1]
	ADT (30 cm)	NP10EO	96h	F, N	mortality	LC_{50}	2500	NR	NR	NR	15-17	U	Swedmark et al. 1971
	ADT (30 cm)	NP10EO	96h	F, N	mortality	LC_{50}	6000	NR	NR	NR	6-8	U	Swedmark et al. 1971
Pleuronectes flesus (flounder)	ADT	NP10EO	96h	F, N	mortality	LC_{50}	3000	NR	NR	NR	15-17	U	Swedmark et al. 1971
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP12EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	7500	7.95	93-94% saturation	NR	12	2	Miossec and Bocquéné 1986
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP17EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	95000	7.84	93-96% saturation	NR	12	2	Miossec and Bocquéné 1986
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP20EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	970000	7.9	96-98% saturation	NR	12	2	Miossec and Bocquéné 1986

APPENDIX IIf (continued). Toxicity of nonylphenol ethoxylates to marine fish through water column exposure.

Species (Common name)	Life stage	Chemical	Dur.*	Test type	Effect	Endpoint	Conc. ($\mu g \cdot L^{-1}$)	pH	D.O. ($mg \cdot L^{-1}$)	Salinity (‰)	Temp. (°C)	Evaluation Rank	Reference
Anguilla anguilla (European eel) - spawn in saltwater	post-LAR (elvers) (70.7 mm)	NP30EO	24h exposure, 24h recovery	S, M	mortality	LC_{50}	$>1 \times 10^{7}$	NR	NR	NR	12	2	Miossec and Bocquéné 1986

NR = not reported
Life Stage (at start of assay): ADT = adult, JUV = juvenile, NEO = neonates, EMB = embryo
* duration reported in hours (h)
Test type: S = static, R = static-renewal, F = flow-through (continuous or intermittent), IPS = single intraperitoneal injection, IPR = repeated intraperitoneal injections
M = measured concentrations throughout experiment, N = nominal concentration
Conc. = concentration; D.O. = dissolved oxygen; Temp. = temperature
Evaluation Rank: 1 = primary, 2 = secondary, U = unacceptable
[1] As cited in Servos et al. 2000

APPENDIX III

Toxicity of nonylphenol to sediment-dwelling life

Appendix IIIa. Toxicity of nonylphenol to freshwater life through spiked-sediment exposure.

Organism	Life Stage	Endpoint	NP ($mg \cdot kg^{-1}$ dw)	Temperature (°C) pH % TOC	Study Conditions	Reference
LETHAL TESTS						
Chironomus tentans (midge)	larvae	14-d exposure, 10% mortality, not toxic (NOEC)	20.1	19 to 21 5.5 (sediment) 1.27	- based on standard ASTM and USEPA test methods - clean, flow-through water - system equilibrated for 1 day	England and Bussard 1993
	larvae	14-d exposure, 31% mortality, toxic (LOEC)	34.2	19 to 21 5.5 (sediment) 1.27	- based on standard ASTM and USEPA test methods - clean, flow-through water - system equilibrated for 1 day	England and Bussard 1993
Rana catesbiana (bullfrog)	tadpole	30-d exposure, 10% mortality, not toxic (NOEC)	155.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d LC_{50}, (toxic)	260.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d exposure, 85% mortality, toxic (LOEC)	390.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
SUB-LETHAL TESTS						
Chironomus tentans (midge)	larvae	14-d exposure, 6% growth reduction, not toxic (NOEC)	20.1	19 to 21 5.5 (sediment)	- based on standard ASTM and USEPA test methods - clean, flow-through water - system equilibrated for 1 day	England and Bussard 1993
	larvae	14-d exposure, 27% growth reduction, toxic (LOEC)	34.2	19 to 21 5.5 (sediment)	- based on standard ASTM and USEPA test methods - clean, flow-through water - system equilibrated for 1 day	England and Bussard 1993

Appendix IIIa (continued). Toxicity of nonylphenol to freshwater life through spiked-sediment exposure.

Organism	Life Stage	Endpoint	NP ($mg \cdot kg^{-1}$ dw)	Temperature (°C) pH % TOC	Study Conditions	Reference
Rana catesbiana (bullfrog)	tadpole	30-d exposure, 10% sublethal effects (lethargy, hemmorhaging, tail lesions), not toxic (NOEC)	155.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d exposure, 25% growth reduction, not toxic (NOEC)	155.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d EC_{50} for growth and other sublethal effects	220.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d exposure, 29% growth reduction, toxic (LOEC)	390.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992
	tadpole	30-d exposure, 100% sublethal effects (lethargy, hemmorhaging, tail lesions), toxic (LOEC)	390.0	17.1 to 18.8 6.9 to 8.3 (water) 0.05	- based on USEPA test methods - clean, flow-through water - system equilibrated for 2.5 hrs	Ward and Boeri 1992

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
LC_{50} = median lethal concentration
EC_{50} = median effective concentration
dw = dry weight

Appendix IIIb. Toxicity of nonylphenol to marine life through spiked-sediment exposure.

Organism	Life Stage	Endpoint	NP ($mg \cdot kg^{-1}$ dw)	Temperature (°C) pH % TOC	Study Conditions	Reference
LETHAL TESTS						
Ampelisca abdita (amphipod)	juvenile 0.7-1.2 mm	10-d LC_{50} (toxic)	98.7	20 NR NR	- based on USEPA test methods - 29 to 32 $g \cdot L^{-1}$ seawater - aeration of water column	Fay et al. 2000
	juvenile 0.7-1.2 mm	10-d LC_{100} (toxic)	243	20 NR NR	- based on USEPA test methods - 29 to 32 $g \cdot L^{-1}$ seawater - aeration of water column	Fay et al. 2000
SUB-LETHAL TESTS						
Capitella sp. I (polychaete)	larvae to adult	78-d exposure, 17% delay in time to first reproduction, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 0% difference in time to first reproduction, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 46% increase in time between reproductive events, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 3% increase in time between reproductive events, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 44% fewer broods per reproductive individual, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 2% more broods per reproductive individual, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 78% fewer eggs per reproductive individual, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 3% fewer eggs per reproductive individual, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 76% fewer eggs per individual, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999

Appendix IIIb (continued). Toxicity of nonylphenol to marine life through spiked-sediment exposure.

Organism	Life Stage	Endpoint	NP ($mg \cdot kg^{-1}$ dw)	Temperature (°C) pH % TOC	Study Conditions	Reference
Capitella sp. I (polychaete)	larvae to adult	78-d exposure, 3% fewer eggs per individual, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 33% decrease in size at maturity, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 16% decrease in size at maturity, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 24% decrease in population growth rate, toxic (LOEC)	174	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999
	larvae to adult	78-d exposure, 0% decrease in population growth rate, not toxic (NOEC)	52	20 NR 7.4	- 26 to 27 $g \cdot L^{-1}$ seawater - renewal of water and sediment weekly	Hansen et al. 1999

NR = not reported
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
dw = dry weight

APPENDIX IV

Toxicity of nonylphenol and its ethoxylates to terrestrial life

APPENDIX IVa. Toxicity of nonylphenol to soil microbial processes.

Organisms / Effect	Endpoint	Concentration ($mg \cdot kg^{-1}$)	Duration*	Soil pH	Test Substrate	Reference
Selected						
CO_2 evolution	NOEC	100	40d	6.8	mixture of sewage sludge compost (digested sludge and sawdust matured for 9 months) & Permian sandstone (75% sand, 13% clay, 12% silt) in ratio 1:2 (dw); adjusted to 60% field moisture capacity with demineralized water	Trocmé et al. 1988
CO_2 evolution (-64%)	LOEC	1000				
CO_2 evolution	NOEC	10	3 mo.	7.3	silt loam, Udic Haploboroll, Calcaric Phaeozem, 13% clay, 42% silt, 55% sand; dried, sieved, stored for 2 years; 3.5% $CaCO_3$	Kirchmann et al. 1991
CO_2 evolution (+26%)	LOEC	500				
N-mineralization	NOEC	500	3 mo.	7.3	silt loam, Udic Haploboroll, Calcaric Phaeozem, 13% clay, 42% silt, 55% sand; dried, sieved, stored for 2 years; 3.5% $CaCO_3$	Kirchmann et al. 1991
(nitrite accumulation)			7d	7.3	silt loam, Udic Haploboroll, Calcaric Phaeozem, 13% clay, 42% silt, 55% sand; dried, sieved, stored for 2 years; 3.5% $CaCO_3$	Kirchmann et al. 1991
Nitrification	NOEC	10				
Nitrification (-35%)	LOEC	500				
Consulted						
Azotobacter spp. (bacteria)			72h	NR	medium: $CaCl_2$, $FeSO_4 \cdot 7\ H_20$, K_2HP0_4, $MgSO_4 \cdot 7\ H_20$, $MnCl_2$, Na_2MoO_4, mannitol, deionized water	Mårtensson and Torstensson 1996
growth	NOEC	18.8 $mg \cdot L^{-1}$				
growth (-95%)	LOEC	37.6 $mg \cdot L^{-1}$				
Azotobacter spp. (bacteria)			72h	NR	medium: $CaCl_2$, $FeSO_4 \cdot 7\ H_20$, K_2HP0_4, $MgSO_4 \cdot 7\ H_20$, $MnCl_2$, Na_2MoO_4, mannitol, deionized water	Mårtensson and Torstensson 1996
N_2-fixation	NOEC	18.8 $mg \cdot L^{-1}$				
N_2-fixation	LOEC	37.6 $mg \cdot L^{-1}$				
Biomass ATP evolution	NOEC	1000	40d	6.8	mixture of sewage sludge compost (digested sludge and sawdust matured for 9 months) & Permian sandstone (75% sand, 13% clay, 12% silt) in ratio 1:2 (dw); adjusted to 60% field moisture capacity with demineralized water	Trocmé et al. 1988
Candida albicans (yeast),	EC_{50}	28 µM (6.2 $mg \cdot L^{-1}$)	8h	NR	yeast nitrogen base medium; incubated on orbital shaker	Karley et al. 1997
decrease in growth rate	EC_{98}	100 µM (22 $mg \cdot L^{-1}$)				
Neurospora crassa			168h	NR	Vogel's minimal media plus 2% (wt/vol) sucrose; incubated on orbital shaker	Karley et al. 1997
growth inhibition	EC_{50}	6 µM (1.3 $mg \cdot L^{-1}$)	(7d)			
growth inhibition	EC_{100}	100 µM (22 $mg \cdot L^{-1}$)				
Neurospora crassa			10 min.	5.8	buffer: 3,3-dimethylglutarate, $CaCl_2$, glucose, KOH	Karley et al. 1997
oxygen uptake rate inhibition	EC_{50}	23 µM (5.1 $mg \cdot L^{-1}$)				
oxygen uptake rate inhibition	EC_{60}	100 µM (22 $mg \cdot L^{-1}$)				

APPENDIX IVa (continued). Toxicity of nonylphenol to soil microbial processes.

Organisms / Effect	Endpoint	Concentration ($mg \cdot kg^{-1}$)	Duration *	Soil pH	Test Substrate	Reference
Nitrification	NOEC	10 $g \cdot kg^{-1}$ sludge dw (concentration of sludge/soil not reported)	6h	NR	sandy loam from Flakkebjerg, Denmark; sieved (2 mm); stored at - 20C; activated sludge aerobically stabilized to remove organic contaminants, spiked with NP, mixed with soil	Madsen et al. 1998
Photobacterium phosphoreum (bacteria), bioluminescence	EC_{50}	5.6 $\mu mol \cdot L^{-1}$ (1.2 $mg \cdot L^{-1}$)	30 min.	NR	NR (Microtox test)	Carlson-Ekvall and Morrison 1995

NR = not reported
dw = dry weight
* duration reported in minutes (min.), hours (h), days (d), or months (mo.)

APPENDIX IVb. Toxicity of nonylphenol to soil invertebrates.

Organism	Life stage	Duration*	Effect (% decrease)	Endpoint	Conc. ($mg \cdot kg^{-1}$)	Soil pH	Test substrate	Reference
Selected								
Apporectodea calignosa (earthworm)	ADT	21d	reproduction (cocoon production)	EC_{10} EC_{50}	3.44 13.7	NR	sandy soil from Lundgård site, 89.7% sand, 3.8% silt, 4.3% clay, 2.2% humus, moisture set to 16% of dw	Krogh et al. 1996
	JUV	21d	growth	EC_{50}	23.9	NR	sandy soil from Lundgård site, 89.7% sand, 3.8% silt, 4.3% clay, 2.2% humus, moisture set to 16% of dw	Krogh et al. 1996
	ADT	21d	mortality	EC_{10} EC_{50}	>40 >40	NR	sandy soil from Lundgård site, 89.7% sand, 3.8% silt, 4.3% clay, 2.2% humus, moisture set to 16% of dw	Krogh et al. 1996
Folsomia candida (springtail)	JUV	28d	reproduction	EC_{50}	16 - 30	NR	loamy sand from Jyndevad, Denmark; sieved (2 mm); defaunated; mixed with spiked sludge	Madsen et al. 1998
	JUV	28d	adult survival	LC_{50}	>30	NR	loamy sand from Jyndevad, Denmark; sieved (2 mm); defaunated; mixed with spiked sludge	Madsen et al. 1998
Folsomia fimetaria (springtail)	23-26 day	21d	reproduction	EC_{10} EC_{50}	23.6 65.5	NR	LUFA sand (German standard soil), 89.4% sand, 5.6% silt, 5.1% clay, 3.9% humus	Krogh et al. 1996
Folsomia fimetaria (springtail)	NR	21d	reproduction	EC_{10} EC_{50}	27 39	NR	sandy soil	Holm as cited in Jensen et al. 1997
Folsomia fimetaria (springtail)	NR	21d	reproduction	EC_{10} EC_{50}	48 59	NR	sandy soil, mixed with sludge	Holm as cited in Jensen et al. 1997
Folsomia fimetaria (springtail)	23-26 day	21d	adult survival	LC_{10} LC_{50}	75.1 151.3	NR	LUFA sand (German standard soil), 89.4% sand, 5.6% silt, 5.1% clay, 3.9% humus	Krogh et al. 1996
Consulted								
Folsomia fimetaria (springtail)	23-26 day	21d	reproduction	EC_{50} EC_{10}	44.2 27.2	NR	Lundgård soil, 89-92% sand, 3-4% silt, 3-4% clay, 1-2% humus	Krogh et al. 1997
Folsomia fimetaria (springtail)	23-26 day	21d	reproduction	EC_{50} EC_{10}	58.4 48.7	NR	Lundgård soil, 89-92% sand, 3-4% silt, 3-4% clay, 1-2% humus; mixed with spiked sludge	Krogh et al. 1997
Folsomia fimetaria (springtail)	23-26 day	21d	adult survival	LC_{50} LC_{10}	85.9 42.7	NR	Lundgård soil, 89-92% sand, 3-4% silt, 3-4% clay, 1-2% humus	Krogh et al. 1997
Folsomia fimetaria (springtail)	23-26 day	21d	adult survival	LC_{50}	>100	NR	Lundgård soil, 89-92% sand, 3-4% silt, 3-4% clay, 1-2% humus; mixed with spiked sludge	Krogh et al. 1997

NR = not reported
Life stage (at start of assay): ADT = adult, JUV = juvenile
* duration reported in days (d)
dw = dry weight

APPENDIX IVc. Toxicity of nonylphenol to terrestrial plants.

Organism	Life stage	Duration*	Effect (% decrease)	Endpoint	Conc. ($mg \cdot kg^{-1}$)	Soil pH	Test substrate	Reference
Selected								
Brassica rapa ssp. *rapa* (turnip)	seedling	14d	growth inhibition	EC_5	10	6.1	sandy loam soil, 54.3% sand, 35.7% silt, 9.9% clay; max water-holding capacity 23.2g·100g^{-1} soil)	Günther and Pestemer 1992
Glycine max (soybean)	seedling	21d	growth	NOEC EC_{50}	100 1000	5.8	grit/loam soil	Windeatt and Tapp 1986
Helianthrus rodeo (sunflower)	seedling	21d	growth	NOEC EC_{50}	100 1000	5.8	grit/loam soil	Windeatt and Tapp 1986
Lactuca sativa (lettuce)	seed to seedling	7d 14d	growth growth	EC_{50} EC_{50}	559 625	7.5	agricultural loam, 24% clay, 1.8% organic matter, sieved (4 mm), 25-30% moisture content (i.e. 80% water-holding capacity)	Hulzebos et al. 1993
Sorghum bicolor (sorghum)	seedling	21d	growth	NOEC EC_{50}	100 1000	5.8	grit/loam soil	Windeatt and Tapp 1986
Consulted								
Atriplex hortensis (garden orache)	intact plant	4d	reduced dry weight yield	EC_{50}	0.01-0.05	NR	grown aseptically in nutrient medium	Bokern et al. 1998
	cell culture	7-14d	reduction of plant cell growth	EC_{50}	22-110 $mg \cdot L^{-1}$ (100-500 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Beta vulgaris var. *altissima* L. cv. Bush Mono Leaves (sugarbeet)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	2.2-11 $mg \cdot L^{-1}$ (10-50 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Beta vulgaris var. *esculenta* L. cv. Monogold (beet)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	22-110 $mg \cdot L^{-1}$ (100-500 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Betula papyrifera Marsh (white birch)	seedling roots	9-11d	electrical conductivity (ion leakage)	EC_{50}	5 $mg \cdot L^{-1}$	NR	media bathing roots	Weinberger and Greenhalgh 1984
	seeds	21d	reduction in sprout length	EC_{85}	5-10 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Greenhalgh 1984
	seeds	6d	germination	NOEC	10 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Greenhalgh 1984
	seedlings	21d	reduced sprout growth	EC_{80}	20 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Vladut 1981
	seeds	21d	enhanced germination	EC_{26}	20 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Vladut 1981
	seeds	21d	reduction in percent germination	EC_{26}	100 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Vladut 1981

APPENDIX IVc (continued). Toxicity of nonylphenol to terrestrial plants.

Organism	Life stage	Duration*	Effect (% decrease)	Endpoint	Conc. ($mg \cdot kg^{-1}$)	Soil pH	Test substrate	Reference
Chenopodium quinoa (quinoa)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	2.2-11 $mg \cdot L^{-1}$ (10-50 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Chenopodium rubrum (red goosefoot)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	11-22 $mg \cdot L^{-1}$ (50-100 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Daucus carota (carrot)	cell culture	48h	growth (dry weight)	NOEC	1 mM	NR	cell culture medium	Harms 1992
	cell culture	7-14d	reduction of plant cell growth	EC_{50}	>220 $mg \cdot L^{-1}$ (>1000 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Glycine max (soybean)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	>220 $mg \cdot L^{-1}$ (>1000 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Hordeum vulgare (barley)	cell culture	48h	growth (dry weight)	LOEC NOEC	0.5 mM 0.1 mM	NR	cell culture medium	Harms 1992
	cell culture	7-14d	reduction of plant cell growth	EC_{50}	22-110 $mg \cdot L^{-1}$ (100-500 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
Lactuca sativa (lettuce)	seed to seedling	21d	growth	EC_{50}	>0.1, <0.32	NR	for germination: perlite saturated with nutrient solution; after germination 5 seedlings with roots >3cm long transferred to nutrient solution only for growth test	Hulzebos et al. 1993
	cell culture	10d	reduction of plant cell growth	EC_{50}	110-220 $mg \cdot L^{-1}$ (500-1000 µM)	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 $mg \cdot L^{-1}$ 6-benzylaminopurine; sucrose 20 $g \cdot L^{-1}$	Bokern and Harms 1997
Lepidium sativum (garden cress)	seeds	72h	inhibition of radicle elongation	EC_{50}	6400 $mg \cdot L^{-1}$	NR	on filter paper in petri dishes	Günther and Pestemer 1992
Lupinus hartwegii (Hartweg's bluebonnet)	cell culture	7d	reduction of plant cell growth	EC_{50}	11-22 $mg \cdot L^{-1}$ (50-100 µM)	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 mM KH_2PO_4	Bokern and Harms 1997
	root culture	7d	growth inhibition	EC_{50}	0.1 mM	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 mM KH_2PO_4; cultured aseptically	Bokern et al. 1998

APPENDIX IVc (continued). Toxicity of nonylphenol to terrestrial plants.

Organism	Life stage	Duration*	Effect (% decrease)	Endpoint	Conc. ($mg \cdot kg^{-1}$)	Soil pH	Test substrate	Reference
Lupinus polyphyllus (bigleaf lupine)	root culture	7d	growth inhibition	EC_{50}	>1 mM	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 mM KH_2PO_4; cultured with microorganisms	Bokern et al. 1998
	cell culture	7d	reduction of plant cell growth	EC_{50}	>220 $mg \cdot L^{-1}$ (>1000 µM)	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 mM KH_2PO_4	Bokern and Harms 1997
	root culture	7d	growth inhibition	EC_{50}	>1 mM	NR	MS medium + 2 $mg \cdot L^{-1}$ 2,4-D + 0.25 $mg \cdot L^{-1}$ kinetine + 1 mM KH_2PO_4; cultured aseptically	Bokern et al. 1998
Lycopersicon esculentum (tomato)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	11-22 $mg \cdot L^{-1}$ (50-100 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997
	cell culture	48h	growth (dry weight)	LOEC NOEC	0.1 mM 0.05 mM	NR	cell culture medium	Harms 1992
Pennisetum americanum Rich. (pearl millet)	cell culture	9d	reduction of plant cell growth	EC_{50}	11-22 $mg \cdot L^{-1}$ (50-100 µM)	NR	MS medium + 5 $mg \cdot L^{-1}$ 2,4-dichlorophenoxyacetic acid	Bokern and Harms 1997
Pinus banksiana (jack pine)	seeds	14d	reduction in sprout length	EC_{60}	5-10 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Greenhalgh 1984
	seeds	NR	germination	NOEC	10 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Greenhalgh 1984
	seedling roots	9-11d	electrical conductivity (ion leakage)	EC_{50}	10 $mg \cdot L^{-1}$	NR	media bathing roots	Weinberger and Greenhalgh 1984
	seeds	14d	reduction in percent germination	EC_{60}	100 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Vladut 1981
	seedlings	14d	reduced sprout growth	EC_{20}	100 $mg \cdot L^{-1}$	NR	no soil – seeds in petri dishes with NP	Weinberger and Vladut 1981
Triticum aestivum (winter wheat)	cell culture	7-14d	reduction of plant cell growth	EC_{50}	22-110 $mg \cdot L^{-1}$ (100-500 µM)	NR	B-5 or MS medium; cultivated on shaker at 120 rpm	Bokern and Harms 1997

NR = not reported
* duration reported in hours (h) or days (d)
Conc. = concentration

APPENDIX IVd. Toxicity of nonylphenol to terrestrial vertebrates.

Organism	Effect (% effect relative to control)	Endpoint	Diet Concentration ($mg \cdot kg^{-1}$)	Average Dose ($mg \cdot kg^{-1}$ BW$\cdot d^{-1}$)	Exposure Period	Reference
Selected						
Rattus norvegicus	**F0 generation:**					Chapin et al. 1999
Sprague Dawley	decreased litter size (9%)	EC	2000	88-185	8 weeks	
(Norway rat)	increased male kidney weight (7%)	EC	650	28-63	11weeks	
	decreased female body weight (9%)	EC	2000	88-185	11 weeks	
	F1 generation:					
	decreased female body weight (7%)	EC	650	30-108	21 weeks	
	earlier vaginal opening (47%)	EC	650	30-108	21 weeks	
	increased pup vagina/uterus relative weight (14%)	EC	650	30-108	21 weeks	
	increased estrous cycle length (14%)	EC	2000	100-353	21 weeks	
	increased male relative kidney weight (20%)	EC	2000	100-353	21 weeks	
	increased female relative kidney weight (11%)	EC	2000	100-353	21 weeks	
	decreased adult ovary weight (19%)	EC	2000	100-353	21 weeks	
	decreased adult testis weight (6%)	EC	2000	100-353	21 weeks	
	decreased adult male body weight (9%)	EC	2000	100-353	21 weeks	
	F2 generation:					
	increased gestation length (2%)	EC	200	10-35	21 weeks	
	sooner to vaginal opening	EC	650	31-115	21 weeks	
	decreased sperm density (8%)	EC	650	31-115	21 weeks	
	increased male relative kidney weight (9%)	EC	650	31-115	21 weeks	
	decreased ovary weight (12%)	EC	650	31-115	21 weeks	
	decreased adult male body weight (8%)	EC	650	31-115	21 weeks	
	increased estrous cycle length (18%)	EC	2000	109-360	21 weeks	
	increased male pup weight adjusted for litter size	EC	2000	109-360	21 weeks	
	decreased number of spermatids (13%)	EC	2000	109-360	21 weeks	
	increased relative kidney weight in female pups (9%)	EC	2000	109-360	21 weeks	
	decreased adult female body weight (10%)	EC	2000	109-360	21 weeks	
	F3 generation:					
	decreased adult female body weight (10%)	EC	650	NR	21 weeks	
	sooner to vaginal opening	EC	650	NR	21 weeks	
	decreased adult epididymis weight (13%)	EC	650	NR	21 weeks	
	decreased adult male body weight (7%)	EC	2000	NR	21 weeks	
	decreased adult ovary weight	EC	2000	NR	21 weeks	
	reproductive effects	NOAEL	200	NR	21 weeks/ generation	

APPENDIX IVd (continued). Toxicity of nonylphenol to terrestrial vertebrates.

Organism	Effect (% effect relative to control)	Endpoint	Diet Concentration ($mg \cdot kg^{-1}$)	Average Dose ($mg \cdot kg^{-1}$ BW$\cdot d^{-1}$)	Exposure Period	Reference
Long Evans rat	increased uterine weight in prepubertal females (10%)	NOEC	25	NR	3 days	Laws et al. 2000
	increased uterine weight in prepubertal females (65%)	LOEC	50	NR	3 days	
	increased uterine weight in ovariectomized females (10%)	NOEC	10	NR	3 days	
	increased uterine weight in ovariectomized females (50%)	LOEC	50	NR	3 days	
	sooner to vaginal opening (5%)	NOEC	25	NR	15 days	
	sooner to vaginal opening (16%)	LOEC	50	NR	15 days	
	disruption of estrous cyclicity (29%)	NOEC	50	NR	25 days	
	disruption of estrous cyclicity (35%)	LOEC	100	NR	25 days	
Noble rat	increased uterine weight in ovariectomized females	LOEC	NR	75	3 days	Odum et al. 1999
Consulted						
Rattus norvegicus Sprague Dawley (Norway rat)	reduced body weight (7%)	EC	2000	129-149	90 days	Cunny et al. 1997
	reduced body weight gain (10-16%)	EC	2000	129-149		
	mortality	NOEC	2000	129-149		
	increased male kidney weight	LOEC	2000	129-149		
	all adverse subchronic effects	NOAEL	650	45-50		
	decreased seminiferous tubule diameter	LOEC	100	NR	10 weeks	De Jager et al. 1999a
	decreased epididymal mass and ratio	LOEC	250	NR	10 weeks	
	decreased testicular mass, testis ratio, and sperm count	LOEC	400	NR	10 weeks	
	decreased body mass, testicular mass, seminiferous tubule diameter of offspring	LOEC	100	NR	prenatal to 10 weeks after weaning	De Jager et al. 1999b
	increased testis ratio, decreased epididymal mass, decreased sperm count in offspring	LOEC	250	NR		

NR = not reported

APPENDIX IVe. Toxicity of nonylphenol ethoxylates to terrestrial organisms.

Organism	Life Stage	Chemical (trade name)	Duration	Effect (% decrease)	Endpoint	Concentration	Soil pH	Test substrate	Reference
Bacteria/Fungi									
Candida albicans (unicellular fungus)	yeast form	NP1EO	8h	decrease in growth rate	NOEC LOEC	100 µM 200µM	NR	yeast nitrogen base medium; incubated on orbital shaker	Karley et al. 1997
Nectria galligena	NR	t-NP9.5EO (Triton N101)	NR	growth inhibition	EC_{42}	500 $\mu g \cdot L^{-1}$	NR	nutrient media	Hislop et al. 1977
Trichoderma viride	NR	t-NP9.5EO (Triton N101)	NR	growth inhibition	EC_{80}	500 $\mu g \cdot L^{-1}$	NR	nutrient media	Hislop et al. 1977
Rhizobium trifolii (N-fixing bacteria)	NR	4-t-NPnEO (PP 222)	35d	growth (reduced colony diameter)	EC_{50}	1000 $mg \cdot L^{-1}$	6.8	agar medium	Fisher et al. 1978
Plants									
Phaseolus vulgaris (green beans)	started from seed	NPnEO (DME)	56d	reduced dry weight yield	NOEC	12.3 $g \cdot 1.8\ kg^{-1}$ soil (=0.00683 $mg \cdot kg^{-1}$)	6.2	Dagor silt loam; 10% clay; fine-loamy, mixed, mesic Cumulic Haploxerolls formed from calcareous sandstones; top 50 cm; air-dried, sieved (0.5 cm)	Miller et al. 1980
Zea mays var. *saccharata* (sweet corn)	started from seed	NPnEO (DME)	56d	reduced dry weight yield	NOEC	12.3 $g \cdot 1.8\ kg^{-1}$ soil (=0.00683 $mg \cdot kg^{-1}$)	6.2	Dagor silt loam; 10% clay; fine-loamy, mixed, mesic Cumulic Haploxerolls formed from calcareous sandstones; top 50 cm; air-dried, sieved (0.5 cm)	Miller et al. 1980
Glycine max (soya)	seedling	NP2EO	21d	growth	NOEC EC_{50}	10 1000	5.8	grit/loam soil	Windeatt and Tapp 1986
Helianthus rodeo (sunflower)	seedling	NP2EO	21d	growth	NOEC EC_{50}	100 1000	5.8	grit/loam soil	Windeatt and Tapp 1986
Sorghum bicolor (sorghum)	seedling	NP2EO	21d	growth	NOEC EC_{50}	10 1000	5.8	grit/loam soil	Windeatt and Tapp 1986

APPENDIX IVe (continued). Toxicity of nonylphenol ethoxylates to terrestrial organisms.

Organism	Life Stage	Chemical (trade name)	Duration *	Effect (% decrease)	Endpoint	Concentration	Soil pH	Test substrate	Reference
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP4EO (Synperonic)	24h	potassium leakage	ca. 22% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP6EO (Synperonic)	24h	potassium leakage	ca. 58% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP8EO (Synperonic)	24h	potassium leakage	ca. 82% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP10EO (Synperonic)	24h	potassium leakage	ca. 80% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP12EO (Synperonic)	24h	potassium leakage	ca. 78% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Vicia faba cv. Maris Bead (bean)	leaf discs (3rd & 4th leaf pair), 21-28d plants	NP20EO (Synperonic)	24h	potassium leakage	ca. 47% of total	0.1% w/v	NR	leaf discs exposed to toxicant solution only	Silcox and Holloway 1986
Trifolium repens (white clover)	seedling	4-t-NPnEO (PP 222)	84d	growth (plant weight)	LOEC NOEC	5000 1000	7	sterile John Innes compost, innoculated with *Rhizobium trifolii*	Fisher et al. 1978

NR = not reported
* duration reported in hours (d) or days (d)
w/v = weight per volume